

Premium
Research Tools
2018



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about highQu

highQu assists life science by fueling it with research tools of premium quality.

Our dedication to the molecular biology field has enabled us to deliver outstanding reagents to the life science market. In our commitment to science, we combine excellence with simplicity in order to provide the solutions that scientists constantly seek. We support researchers whose professionalism makes science come alive.

highQu honors people who have a higher respect for humanity itself than for anything invented by humans.

We are devoted to deliver intelligent products and services that render a better life for all.

This is our interpretation of life science: we are people, we serve life.

Professionally simple

- We offer premium tools for researchers
- We keep our word
- We contribute to your success

highQu is determined to supply professionally simple life science research products – minimum optimization, fast and easy procedures, short protocols, outstanding performance in both professional and student hands.

Founded in 2013, highQu has already entered many research laboratories in Europe and worldwide. Our free samples of premium quality tools for end-point PCR, real-time PCR (qPCR), RT-PCR, electrophoresis and other molecular biology applications convince numerous scientists to stay with highQu products that provide one of the best price-performance ratios in the market supported by our outstanding customer service.

We know you have a world full of choices, thus we are especially thankful for trusting and selecting highQu.

Your highQu team

Featured in 2018

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Direct Hot Start PCR	SampleIN™ Direct PCR Kit	Low background high yield PCR for direct gel loading: No need to add loading dye after the PCR and no need to extract DNA before PCR.	41
PCR Detection	PCRbeam™ Fast PCR Detection Kit	Sensitive PCR product detection in the tube, without electrophoresis.	52
DNA/RNA Electrophoresis	StainIN™ RED Nucleic Acid Stain	Non-mutagen, safer, economical alternative to ethidium bromide. 2X more sensitive in-gel staining of DNA and RNA for UV detection.	62
DNA/RNA Electrophoresis	StainIN™ GREEN Nucleic Acid Stain	Non-mutagen, safer, economical alternative to ethidium bromide. 4X more sensitive in-gel staining of DNA and RNA for UV and Blue light detection.	63
Protein Electrophoresis	CozyXL™ Prestained Protein Ladder NEW	High range protein ladder for protein electrophoresis and Western blots. Unique high MW 310 kDa protein band.	65

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QPP0401	ORA™ SEE qPCR Probe Mix	2X	200	r of 20 µl	10
QPP0405	ORA™ SEE qPCR Probe Mix	2X	1000	r of 20 µl	
QPP0201	ORA™ qPCR Probe ROX L Mix	2X	200	r of 20 µl	11
QPP0205	ORA™ qPCR Probe ROX L Mix	2X	1000	r of 20 µl	
QPP0501	ORA™ SEE qPCR Probe ROX L Mix	2X	200	r of 20 µl	12
QPP0505	ORA™ SEE qPCR Probe ROX L Mix	2X	1000	r of 20 µl	
QPP0301	ORA™ qPCR Probe ROX H Mix	2X	200	r of 20 µl	13
QPP0305	ORA™ qPCR Probe ROX H Mix	2X	1000	r of 20 µl	
QPP0601	ORA™ SEE qPCR Probe ROX H Mix	2X	200	r of 20 µl	14
QPP0605	ORA™ SEE qPCR Probe ROX H Mix	2X	1000	r of 20 µl	
QPD0101	ORA™ qPCR Green ROX L Mix	2X	200	r of 20 µl	15
QPD0105	ORA™ qPCR Green ROX L Mix	2X	1000	r of 20 µl	
QPD0501	ORA™ SEE qPCR Green ROX L Mix	2X	200	r of 20 µl	16
QPD0505	ORA™ SEE qPCR Green ROX L Mix	2X	1000	r of 20 µl	
QPD0201	ORA™ qPCR Green ROX H Mix	2X	200	r of 20 µl	17
QPD0205	ORA™ qPCR Green ROX H Mix	2X	1000	r of 20 µl	
QPD0401	ORA™ SEE qPCR Green ROX H Mix	2X	200	r of 20 µl	18
QPD0405	ORA™ SEE qPCR Green ROX H Mix	2X	1000	r of 20 µl	
QPD0301	ORA™ qPCR HRM Mix	2X	200	r of 20 µl	19
QPD0305	ORA™ qPCR HRM Mix	2X	1000	r of 20 µl	
QOP0101	1Step RT qPCR Probe Kit	2X & 20X	200	r of 20 µl	23
QOP0105	1Step RT qPCR Probe Kit	2X & 20X	1000	r of 20 µl	
QOP0201	1Step RT qPCR Probe ROX L Kit	2X & 20X	200	r of 20 µl	24
QOP0205	1Step RT qPCR Probe ROX L Kit	2X & 20X	1000	r of 20 µl	
QOP0301	1Step RT qPCR Probe ROX H Kit	2X & 20X	200	r of 20 µl	25
QOP0305	1Step RT qPCR Probe ROX H Kit	2X & 20X	1000	r of 20 µl	
QOD0101	1Step RT qPCR Green ROX L Kit	2X & 20X	200	r of 20 µl	26
QOD0105	1Step RT qPCR Green ROX L Kit	2X & 20X	1000	r of 20 µl	
QOD0201	1Step RT qPCR Green ROX H Kit	2X & 20X	200	r of 20 µl	27
QOD0205	1Step RT qPCR Green ROX H Kit	2X & 20X	1000	r of 20 µl	
PCE0101	ALLin™ Taq DNA Polymerase	5 u/µl	500	u	31
PCE0105	ALLin™ Taq DNA Polymerase	5 u/µl	2500	u	
PCM0201	ALLin™ Red Taq Mastermix	2X	200	r of 50 µl	32
PCM0205	ALLin™ Red Taq Mastermix	2X	1000	r of 50 µl	
PCM0101	ALLin™ Taq Mastermix	2X	200	r of 50 µl	33
PCM0105	ALLin™ Taq Mastermix	2X	1000	r of 50 µl	
PCE0201	Taq DNA Polymerase	5 u/µl	1500	u	34
PCE0202	Taq DNA Polymerase	5 u/µl	3000	u	
HSE0101	ALLin™ Hot Start Taq Polymerase	5 u/µl	500	u	35
HSE0105	ALLin™ Hot Start Taq Polymerase	5 u/µl	2500	u	
HSM0301	ALLin™ HS Red Taq Mastermix	2X	200	r of 50 µl	36
HSM0305	ALLin™ HS Red Taq Mastermix	2X	1000	r of 50 µl	
HSM0201	ALLin™ Hot Start Taq Mastermix	2X	200	r of 50 µl	37
HSM0205	ALLin™ Hot Start Taq Mastermix		1000	r of 50 µl	
HLE0101	ALLin™ RPH Polymerase	5 u/µl	250	u	38
HLE0105	ALLin™ RPH Polymerase	5 u/µl	1250	u	
HLM0101	ALLin™ RPH Mastermix	2X	200	r of 50 µl	39
HLM0105	ALLin™ RPH Mastermix	2X	1000	r of 50 µl	
HLE0201	ALLin™ HiFi DNA Polymerase	2 u/µl	200	u	40
HLE0205	ALLin™ HiFi DNA Polymerase	2 u/µl	1000	u	

Product Index

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DPK0105	SampleIN™ Direct PCR Kit		400	r of 50 µl	
RTK0201	1Step RT PCR Kit	2X & 20X	100	r of 50 µl	45
RTM0301	HighScriber™ Reverse Transcriptase Mix	20X	10000	u	46
RTM0305	HighScriber™ Reverse Transcriptase Mix	20X	50000	u	
RTK0101	qScriber™ cDNA Synthesis Kit	5X & 20X	25	r of 20 µl	47
RTK0104	qScriber™ cDNA Synthesis Kit	5X & 20X	100	r of 20 µl	
UDG0101	UDGin™ PCR Cleaner Mix	20X	0.5	ml	51
PDK0101	PCRbeam™ Fast PCR Detection Kit		50	tests	52
NUM0101	25 mM dNTP Mix	25 mM each	1	ml	53
NUM0201	10 mM dNTP Mix	10 mM each	1	ml	53
NUS0101	100 mM dNTP Set	100 mM	4 x 0.25	ml	53
WAT0110	PCR Water		10 x 1	ml	54
RLK0101	Rally™ Rapid Ligation Kit	1 r/µl	40	r of 20 µl	57
RLK0105	Rally™ Rapid Ligation Kit	1 r/µl	200	r of 20 µl	
HER0101	HighEnd™ Repair Kit	1 r/µl	40	r of 25 µl	58
HER0105	HighEnd™ Repair Kit	1 r/µl	200	r of 25 µl	
DNL0102	Take5™ 1kb DNA Ladder	5 µl/appl.	200	appl. (5 µl)	61
DNL0202	Take5™ 100 bp DNA Ladder	5 µl/appl.	200	appl. (5 µl)	61
DNL0302	Take5™ 50 bp DNA Ladder	5 µl/appl.	200	appl. (5 µl)	61
DNL0402	Take5™ HR DNA Ladder	5 µl/appl.	200	appl. (5 µl)	61
NAS0101	StainIN™ RED Nucleic Acid Stain	20000X	1	ml	62
NAS0201	StainIN™ GREEN Nucleic Acid Stain	20000X	1	ml	63
PRL0102	Cozy™ Prestained Protein Ladder	5 µl/appl.	200	appl. (5 µl)	65
PRL0202	CozyHi™ Prestained Protein Ladder	5 µl/appl.	200	appl. (5 µl)	65
PRL0302	CozyXL™ Prestained Protein Ladder	5 µl/appl.	200	appl. (5 µl)	65

Trademarks of highQu

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 CozyHi
 CozyXL
 HighEnd
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 HighScriber
 qScriber
 ORA
 PCRbeam
 professionally simple
 Rally
 UDGin
 SampleIN
 StainIN
 Take5

highQu qPCR master mixes are well-known for their excellent performance and ease of use with minimum optimization required. Supplied with PCR water, optimized for both common and fast cycling workflows, they convince by their early Ct values, and provide reproducible results.

Want to try one? Order a sample today at
www.highqu.com/samplerequest

Bulk orders are welcome at order@highQu.com

qPCR & HRM Master Mixes



qPCR Selection: Instrument Compatibility of Probe & Dye-based ORA™ qPCR Mixes

Probe-based qPCR			Green dye-based qPCR		
ORA™ (SEE) qPCR Probe	ORA™ (SEE) qPCR Probe ROX L	ORA™ (SEE) qPCR Probe ROX H	Instruments	ORA™ (SEE) qPCR Green ROX L	ORA™ (SEE) qPCR Green ROX H
<i>page 9-10</i>	<i>page 11-12</i>	<i>page 13-14</i>		<i>page 15-16</i>	<i>page 17-18</i>
●	●		Analytic Jena: qTOWER BioRad: Opticon®, Opticon®2, Chromo4™, MiniOpticon™, CFX96™, CFX384™ Cepheid: SmartCycler® BJS: Xpress® Illumina: Eco Eppendorf: Mastercycler® ep realplex, Mastercycler® realplex 2S Hain Lifescience: FluoroCycler®96 QIAGEN: Rotor-Gene®Q, Rotor-Gene® 6000, Rotor-Gene® 3000 Roche Applied Science: LightCycler®480, LightCycler®96, LightCycler®Nano Takara: Thermal Cycler Dice® Thermo Fisher Scientific: Piko Real® Techne: PrimeQ, Quantica®	●	
	●		Agilent: Mx3000P®, Mx3005P®, Mx4000P® Fluidigm: BioMark™ Life Technologies: 7500, 7500 FAST, Viia™7, QuantStudio™ 12K Flex	●	
		●	Life Technologies: 7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™, StepOnePlus™		●
●			BioRad: iCycler®, MyiQ™, iQ™5		

High Resolution Melting Analysis Selection: Instrument Compatibility of ORA™ qPCR HRM Mix (*page 19*)

Life Technologies:	7500, 7500 FAST, 7900, 7900HT FAST, 7900HT, Viia™7, QuantStudio™ 12K Flex
BioRad:	CFX96™, CFX384™
Eppendorf:	Mastercycler® ep realplex Mastercycler® realplex 2S
Illumina:	Eco
QIAGEN:	Rotor-Gene®Q, Rotor-Gene® 6000, Rotor-Gene® 3000
Roche Applied Science:	LightCycler®480, LightCycler®96, LightCycler®Nano

ORA™ qPCR Probe Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0101	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Probe Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, ROX is not included.
QPP0105	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR Probe Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration. See the selection table on page 8.

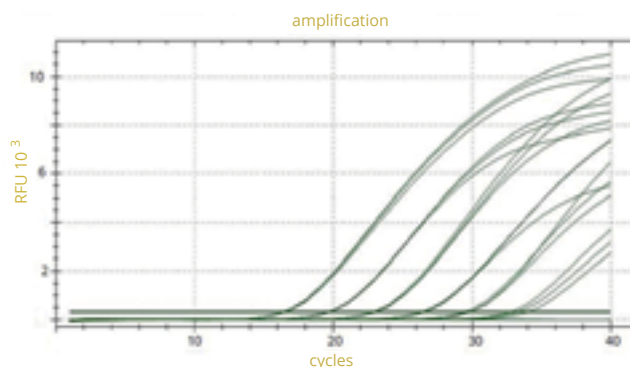
BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

PERFORMANCE

ORA™ qPCR Probe Mix provides high sensitivity 100% efficiency qPCR from 10 copies of the target: TaqMan™ probe amplification traces from plasmid dilution series of 1x10⁶ copies to 10 copies of DNA.

95 oC 2 m, 40 x 95 oC 10 s & 60 oC 15 s, Biorad CFX. Human gene ACVR2B.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0401	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Probe Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer; ROX is not included.
QPP0405	1000 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Probe Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization. ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.

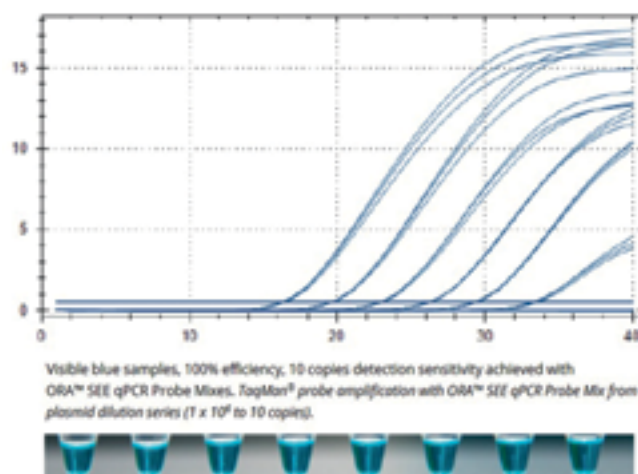
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.

See the selection table on page 8.

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Inert blue dye for a better sample visibility and tracking

PERFORMANCE



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ SEE qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

ORA™ qPCR Probe ROX L Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0201	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Probe ROX L Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, low ROX concentration.
QPP0205	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR Probe ROX L Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration. See the selection table on page 8.

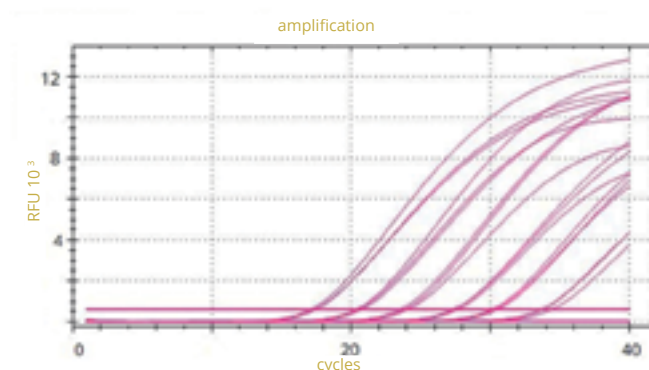
BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

PERFORMANCE

ORA™ qPCR Probe Mix provides high sensitivity 100% efficiency qPCR from 10 copies of the target: TaqMan® probe amplification traces from plasmid dilution series of 1x10⁶ copies to 10 copies of DNA.

95 °C 2 m, 40 x 95 °C 10 s & 60 °C 15 s, Biorad CFX. Human gene LIMK1.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0501	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Probe ROX L Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer; low ROX concentration.
QPP0505	1000 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Probe ROX L Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR on instruments calibrated with low ROX conc.
- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization. ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.

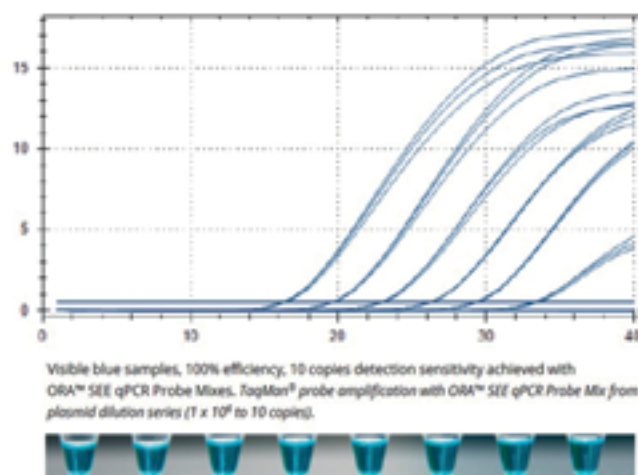
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.

See the selection table on page 8.

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Inert blue dye for a better sample visibility and tracking

PERFORMANCE



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ SEE qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

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ORA™ qPCR Probe ROX H Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0301	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Probe ROX H Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, high ROX concentration.
QPP0305	200 r of 20 µl	10 x 1 ml - ORA™ qPCR Probe ROX H Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.

See the selection table on page 8.

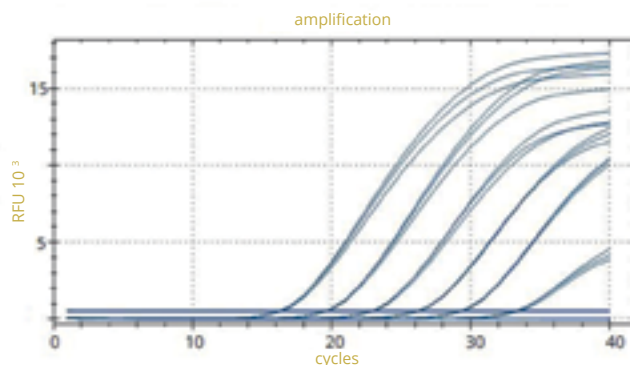
BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

PERFORMANCE

ORA™ qPCR Probe Mix provides high sensitivity 100% efficiency qPCR from 10 copies of the target: TaqMan® probe amplification traces from plasmid dilution series of 1x10⁶ copies to 10 copies of DNA.

95 °C 2 m, 40 x 95 °C 10 s & 60 °C 15 s, Biorad CFX. Human gene ACVR1B.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.

- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

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CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPP0601	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Probe ROX H Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer; high ROX concentration.
QPP0605	200 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Probe ROX H Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR on instruments calibrated with high ROX conc.
- qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of gDNA, cDNA, viral DNA, low copy number genes, gene expression analysis

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates, in multiplexing and guaranty rapid extension with early Ct values with minimum or no optimization. ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.

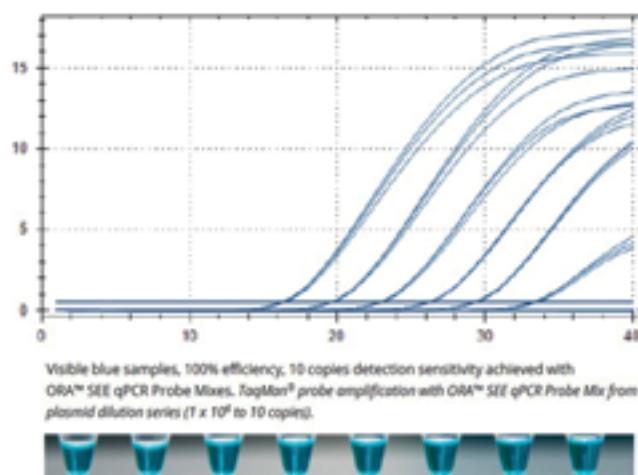
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration.

See the selection table on page 8.

BENEFITS

- Universal - both standard and fast cycling, all probe qPCR assays, GC or AT rich templates
- Excellent for both single-plex & multiplexing
- Rapid extension, early Ct
- Inert blue dye for a better sample visibility and tracking

PERFORMANCE



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

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ORA™ qPCR Green ROX L Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0101	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Green ROX L Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, low ROX concentration.
QPD0105	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR Green ROX L Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR from gDNA, cDNA, viral DNA, low copy number genes
- Relative gene expression analysis, absolute quantification
- qPCR on instruments calibrated with low ROX conc.
- qPCR assays based on fluorescence of intercalating dye

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Green Mixes are available in different versions – with low or high ROX concentration.

See the selection table on page 8.

BENEFITS

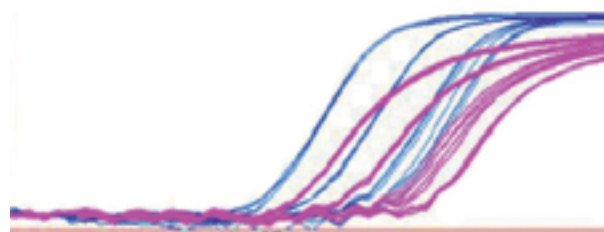
- Universal - standard and fast cycling, GC or AT rich templates
- Highest sensitivity, rapid extension, early Ct values
- Supplied with PCR Water for maximum convenience

PERFORMANCE

ORA™ qPCR Green Mix (blue curves) provides in many cases earlier Ct values compared to competitor mastermixes.

Conditions: 95 °C 2 m, 40 x 95 °C 10 s & 60 °C 15 s, Roche LightCycler® 480.

Amplification of mouse ACTG1 from cDNA dilution series.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

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CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0501	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Green ROX L Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, low ROX concentration.
QPD0505	1000 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Green ROX L Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR from gDNA, cDNA, viral DNA, low copy number genes
- Relative gene expression analysis, absolute quantification
- qPCR on instruments calibrated with low ROX conc.
- qPCR assays based on fluorescence of intercalating dye

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.

ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Green Mixes are available in different versions –with low or high ROX concentration.

See the selection table on page 8.

BENEFITS

- Universal - both standard and fast cycling, GC or AT rich templates
- Highest sensitivity, rapid extension, early Ct
- Supplied with PCR Water for maximum convenience
- Inert blue dye for a better sample visibility and tracking

PERFORMANCE



Visible blue samples, earlier Ct values, superb sensitivity achieved with ORA™ SEE qPCR Green Mixes. Amplification & melt traces of mouse actin gamma-1 housekeeping gene from a cDNA dilution series; ORA™ SEE qPCR Green Mix (purple) and Competitor Mix (black).



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ SEE qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

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ORA™ qPCR Green ROX H Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0201	200 r of 20 µl	2 x 1 ml - ORA™ qPCR Green ROX H Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, high ROX concentration.
QPD0205	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR Green ROX H Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR from gDNA, cDNA, viral DNA, low copy number genes
- Relative gene expression analysis, absolute quantification
- qPCR on instruments calibrated with high ROX conc.
- qPCR assays based on fluorescence of intercalating dye

PRODUCT DETAILS

highQu qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.

Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Green Mixes are available in different versions – with low or high ROX concentration.

See the selection table on page 8.

BENEFITS

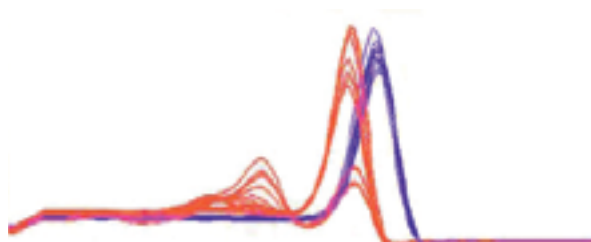
- Universal - standard and fast cycling, GC or AT rich templates
- Highest sensitivity, rapid extension, early Ct values
- Supplied with PCR Water for maximum convenience

PERFORMANCE

ORA™ qPCR Green Mix (blue curves) provides in many cases higher sensitivity compared to competitor mastermixes.

Conditions: 95 °C 2 m, 40 x 95 °C 10 s & 60 °C 15 s, Roche LightCycler® 480.

Amplification of mouse ACTG1 from cDNA dilution series.



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

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CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0401	200 r of 20 µl	2 x 1 ml - ORA™ SEE qPCR Green ROX H Mix, 2X 2 x 1 ml - PCR Water	Mix includes an inert blue dye for better visibility, Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, high ROX concentration.
QPD0405	1000 r of 20 µl	10 x 1 ml - ORA™ SEE qPCR Green ROX H Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR from gDNA, cDNA, viral DNA, low copy number genes
- Relative gene expression analysis, absolute quantification
- qPCR on instruments calibrated with high ROX conc.
- qPCR assays based on fluorescence of intercalating dye

PRODUCT DETAILS

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ORA™ SEE qPCR mixes provide an additional advantage of a simplified tracking of the process, as they are colored with an inert blue dye to make samples much better visible during pipetting and handling.

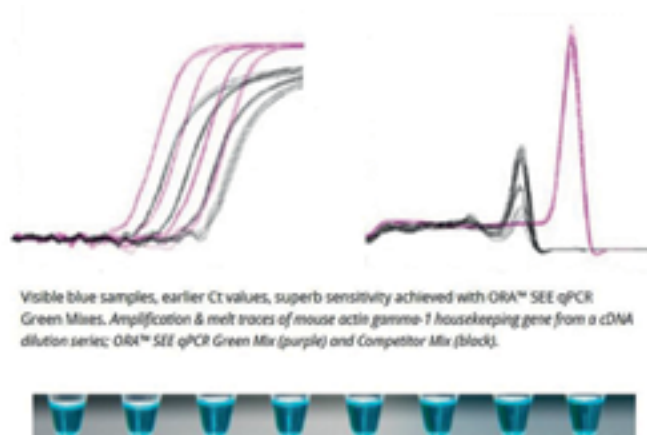
Our mastermixes are supplied with PCR Water to guaranty the best performance. To suit the broad instrument range the ORA™ qPCR Green Mixes are available in different versions –with low or high ROX concentration.

See the selection table on page 8.

BENEFITS

- Universal - both standard and fast cycling, GC or AT rich templates
- Highest sensitivity, rapid extension, early Ct
- Supplied with PCR Water for maximum convenience
- Inert blue dye for a better sample visibility and tracking

PERFORMANCE



PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ SEE qPCR Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument (SYBR® Green or FAM channel) set like:

Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec

- ✓ Follow instrument instructions for melting curve analysis.

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

ORA™ qPCR HRM Mix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0301	200 r of 20 µl	2 x 1 ml - ORA™ qPCR HRM Mix, 2X 2 x 1 ml - PCR Water	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer, proprietary saturating intercalating dye.
QPD0305	1000 r of 20 µl	10 x 1 ml - ORA™ qPCR HRM Mix, 2X 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

High Resolution Melting analysis (HRM):

- Detection of sequence variations
- SNP genotyping
- Methylation analysis
- Mutation scanning

PRODUCT DETAILS

High Resolution Melting analysis (HRM) is a fast and simple technique for identification of DNA sequence variations. It allows identifying single nucleotide differences by detecting minor changes in qPCR melting curves.

highQu ORA™ HRM qPCR Mix includes a proprietary intercalating saturating dye showing no inhibition for PCR. The dye has the same affinity for both AT or GC rich sequences what leads to highest accuracy in genotyping.

The hot-start function in the mix is based on the small molecular inhibitor technology and allows achieving highest sensitivity and specificity under both standard and fast qPCR cycling conditions. The mix provides excellent performance on both AT and GC rich templates and reliable results with minimum or no optimization.

BENEFITS

- Time and costs saving analysis of sequence variations
- Universal - standard or fast cycling, GC or AT rich templates
- Highest sensitivity, no optimization required
- Supplied with PCR Water for maximum convenience

COMPATIBLE INSTRUMENTS

Life Technologies:	7500, 7500 FAST, 7900, 7900HT FAST, 7900HT, ViiA™7, QuantStudio™ 12K Flex
BioRad:	CFX96™, CFX384™
Eppendorf:	Mastercycler® ep realplex Mastercycler® realplex 2S
Illumina:	Eco
QIAGEN:	Rotor-Gene®Q, Rotor-Gene® 6000, Rotor-Gene® 3000
Roche Applied Science:	LightCycler®480, LightCycler®96, LightCycler®Nano

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
cDNA Template or gDNA Template	<100 ng or 1 µg
PCR Water	to 10 µl
ORA™ HRM Mix, 2X	10 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument (SYBR® Green or FAM channel) set like: 	
Initial denaturation	1 cycle: 95°C - 2 min for cDNA, or 1 cycle: 95°C - 3 min for gDNA
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C - 20 - 30 sec
<ul style="list-style-type: none"> ✓ Follow instrument instructions for melting curve analysis. 	

highQu RT qPCR kits are valued for their robust performance and ease of use with minimum optimization required. Supplied with PCR water, optimized for both common and fast cycling workflows, they convince by their early Ct values and by reproducible results they provide.

Want to try one? Order a sample today at
www.highqu.com/samplerequest

Bulk orders are welcome at order@highQu.com

One Step RT qPCR Kits



One Step RT qPCR Selection: Instrument Compatibility for Probe and Dye-based 1Step RT qPCR Kits

Probe-based One-step RT qPCR			Green dye-based One-step RT qPCR		
1Step RT qPCR Probe	1Step RT qPCR Probe ROX L	1Step RT qPCR Probe ROX H	Instruments	1Step RT qPCR Green ROX L	1Step RT qPCR Green ROX H
<i>page 23</i>	<i>page 24</i>	<i>page 25</i>		<i>page 26</i>	<i>page 27</i>
●	●		Analytic Jena: qTOWER BioRad: Opticon®, Opticon®2, Chromo4™, MiniOpticon™, CFX96™, CFX384™ Cepheid: SmartCycler® BJS: Xpress® Illumina: Eco Eppendorf: Mastercycler® ep realplex, Mastercycler® realplex 2S Hain Lifescience: FluoroCycler®96 QIAGEN: Rotor-Gene®Q, Rotor-Gene® 6000, Rotor-Gene® 3000 Roche Applied Science: LightCycler®480, LightCycler®96, LightCycler®Nano Takara: Thermal Cycler Dice® Thermo Fisher Scientific: Piko Real® Techne: PrimeQ, Quantica®	●	
	●		Agilent: Mx3000P®, Mx3005P®, Mx4000P® Fluidigm: BioMark™ Life Technologies: 7500, 7500 FAST, Vii™7, QuantStudio™ 12K Flex	●	
		●	Life Technologies: 7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™, StepOnePlus™		●
●			BioRad: iCycler®, MyiQ™, iQ™5		

1Step RT qPCR Probe Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0101	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Probe Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer 20X concentrated blend of modified MMuLV RT and RNase Inhibitor.
QOP0105	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Probe Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR. qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization. To suit the broad instrument range the 1Step RT qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration. *See the selection table on page 22.*

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC/AT rich templates
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.

Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument set like: 	
Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec
<ul style="list-style-type: none"> ✓ Follow instrument instructions for melting curve analysis. 	

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

Notice to Purchaser: With purchasing of this product, no rights are conveyed with respect to U.S. Patent: 5,928,907 and corresponding patents outside the US.

1Step RT qPCR Probe ROX L Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0201	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Probe ROX L Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, low ROX concentration.
QOP0205	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Probe ROX L Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	20X concentrated blend of modified MMuLV RT and RNase Inhibitor.

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR on instruments calibrated with low ROX conc.
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR. qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT/GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization. To suit the broad instrument range the 1Step RT qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration. *See the selection table on page 22.*

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC/AT rich templates
- Rapid extension, early Ct
- Supplied with PCR Water for maximum convenience

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.

Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument set like: 	
Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec
<ul style="list-style-type: none"> ✓ Follow instrument instructions for melting curve analysis. 	

For optional use, the ROX passive reference dye is premixed within the ROX L and ROX H qPCR Mixes. If the purchaser has an instrument capable of optional ROX detection and wishes to perform the optional normalization of the signal, then the user must select the option in the software.

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1Step RT qPCR Probe ROX H Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOP0301	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Probe ROX H Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, high ROX concentration.
QOP0305	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Probe ROX H Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	20X concentrated blend of modified MMuLV RT and RNase Inhibitor.

Storage: In the dark at -20°C.

APPLICATIONS

- qPCR on instruments calibrated with high ROX conc.
- RT qPCR assays based on specific probes: including TaqMan®, Molecular Beacons, Scorpions™ Probes
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR. qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum optimization.

To suit the broad instrument range the 1Step RT qPCR Probe Mixes are available in three versions – without ROX, with low or high ROX concentration. *See the selection table on page 22.*

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC/AT rich templates
- Rapid extension, early Ct
- PCR Water supplied for maximum convenience

PRECAUTIONS FOR WORK WITH RNA

Take care to prevent RNA from degradation by widely spread and stable RNases. Prepare crude samples and set up reactions in different dedicated areas, use DEPC-treated nuclease-free labware and gloves.

Before the cDNA synthesis, check RNA quality on denaturing agarose gel to be sure you have good quality material.

PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control, no RT Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument set like: 	
Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec
<ul style="list-style-type: none"> ✓ Follow instrument instructions for melting curve analysis. 	

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1Step RT qPCR Green ROX L Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOD0101	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Green ROX L Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, low ROX concentration.
QOD0105	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Green ROX L Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	20X concentrated blend of modified MMuLV RT and RNase Inhibitor.

Storage: In the dark at -20°C.

APPLICATIONS

- Relative gene expression analysis, absolute quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes
- Intercalating dye fluorescence based qPCR on instruments calibrated with low ROX conc.

PRODUCT DETAILS

highQu 1Step RT qPCR mastermixes in combination with a blend of thermostable and extremely active Reverse Transcriptase & advanced RNase Inhibitor (RT Mix) allow for a single step one tube RT qPCR. qPCR mastermixes are based on the small molecular inhibitor technology Hot Start PCR allowing to achieve highest sensitivity and specificity under both standard and fast qPCR cycling conditions. They provide excellent results on both AT and GC rich templates and guaranty rapid extension with early Ct values with minimum or no optimization.

To suit the broad instrument range the 1Step RT qPCR Green Mixes are available in different versions – with low or high ROX concentration. *See the selection table on page 22.*

BENEFITS

- Reverse transcription & qPCR in one tube with highest sensitivity
- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
- Universal Kit for standard and fast cycling, GC/AT rich templates
- Rapid extension, early Ct
- PCR Water supplied for maximum convenience

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PROTOCOL

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- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
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IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
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Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument set like: 	
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1Step RT qPCR Green ROX H Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QOD0201	200 r of 20 µl	2 x 1 ml - 1Step RT qPCR Green ROX H Mix, 2X 2 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 2 x 1 ml - PCR Water	Hot Start Taq, dNTPs at 0.25 mM, optimized buffer, high ROX concentration.
QOD0205	1000 r of 20 µl	10 x 1 ml - 1Step RT qPCR Green ROX H Mix, 2X 10 x 0.2 ml - RT3 Mix, 20X (RNase Inhibitor+RTase) 10 x 1 ml - PCR Water	20X concentrated blend of modified MMuLV RT and RNase Inhibitor.

Storage: In the dark at -20°C.

APPLICATIONS

- Relative gene expression analysis, absolute quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number genes
- Intercalating dye fluorescence based qPCR on instruments calibrated with high ROX conc.

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To suit the broad instrument range the 1Step RT qPCR Green Mixes are available in different versions – with low or high ROX concentration. *See the selection table on page 22.*

BENEFITS

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- Efficient cDNA synthesis ensured by the thermostable Reverse Transcriptase & advanced RNase Inhibitor blend
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- Thaw and keep reagents on ice. Mix well before use.
- Higher amounts of RT3 Mix improve Ct, but primer dimers may appear.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primer	100-400 nM final c.
Forward Primer	100-400 nM final c.
Specific Probe	200 nM final c. (0.4 µl of 10 µM)
Total RNA Template or mRNA Template	1 pg to 1 µg or >0.01 pg
PCR Water	to 10 µl
1Step RT qPCR Mix, 2X	10 µl
RT3 Mix, 20X	1 - 2 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument set like: 	
Reverse Transcription	1 cycle: 40 - 55 °C – 10 min
Initial denaturation	1 cycles: 95°C - 2 min
Denaturation	40 cycles: 95°C - 5 sec
Annealing/extension	40 cycles: 60 - 65°C – 20 - 30 sec
<ul style="list-style-type: none"> ✓ Follow instrument instructions for melting curve analysis. 	

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highQu end-point PCR portfolio provides all you need for both routine and demanding PCR applications. Our engineered Taq combined with an advanced buffer ensures high PCR yield under both standard and fast cycling conditions; on both common and GC rich templates. Proprietary 5X ALLin™ PCR Buffer includes nucleotides, salts and enhancers optimized to ensure best PCR results.

Want to try one of our enzymes or mixes?

Order a sample today at www.highqu.com/samplerequest

Bulk orders are welcome at order@highQu.com

End-point PCR Enzymes & Master Mixes



End-point PCR Selection: Standard, Hot-start, Long, High fidelity, Direct PCR

	Standard			Hot Start		Robust, Long & Hot Start		High Fidelity	Direct
	ALLin™ Taq DNA Polymerase	ALLin™ (RED) Taq Mastermix	Taq DNA Polymerase	ALLin™ Hot Start Taq Polymerase	ALLin™ Hot Start (HS Red) Taq Mastermix	ALLin™ RPH Polymerase	ALLin™ RPH Mastermix	ALLin™ HiFi DNA Polymerase	SampleIN™ Direct PCR Kit
	page 31	page 32-33	page 34	page 35	page 36-37	page 38	page 39	page 40	page 41
Fast cycling	●	●		●	●	●	●	●	●
GC/AT rich PCR	● ●	●		● ●	●	● ●	●	● ●	●
Hot Start				●	●	●	●		●
High sensitivity				● ●	● ●	●	●		●
Fidelity vs Taq	1 X	1 X	1 X	1 X	1 X	5 X	5 X	50 X	1 X
Long PCR	●					● ●	● ●	●	
Max. amplicon	6 kb	6 kb	5 kb	6 kb	6 kb	35 kb	35 kb	10 kb	5 kb
High yields	● ●	● ●	●	●	●	● ●	● ●	●	●
Direct PCR	Colony	Colony	Colony	Colony, blood, urine	Colony, blood, urine	Colony, blood	Colony, blood	Colony	Mouse tail, ear, blood, tissues, swab, hair
Multiplex PCR	●	●		● ●	● ●	●	●		●
Classical PCR			● ●						
Cloning	TA	TA	TA	TA	TA	TA	TA	Blunt	TA
Direct Loading on gels		Red Mix			Red Mix				Red Mix
ALLin™ Buffer* with dNTPs	●			●		●		●	
Mastermix		●			●		●		●

* ALLin™ PCR buffers contain optimal dNTP and magnesium concentrations, PCR enhancers and stabilizers what allows for easier amplification of GC or AT rich templates and for success in fast cycling

ALLin™ Taq DNA Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PCE0101	500 u	500 u - ALLin™ Taq DNA Polymerase, 5 u/μl 4 x 1 ml - 5X ALLin™ PCR Buffer	Enzyme in storage buffer. 1X ALLin™ PCR Buffer contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers.
PCE0105	2500 u	5 x 500 u - ALLin™ Taq DNA Polymerase, 5 u/μl 20 x 1 ml - 5X ALLin™ PCR Buffer	

Storage: In the dark at -20°C.

APPLICATIONS

- Routine PCR up to 6 kb
- Amplification of complex (GC/AT rich) templates
- Colony PCR
- Fast PCR
- TA cloning

PRODUCT DETAILS

highQu ALLin™ Taq DNA Polymerase is the versatile engineered enzyme which in combination with the optimized ALLin™ buffer provides higher success rates in demanding PCR applications like amplification of complex templates, crude sample PCR and fast cycling.

ALLin™ Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, 4.5×10^4 (a number of correct nucleotides incorporated before the first error) and produces A-tailed products suitable for ligating into TA cloning vectors.

For the maximum convenience the 2X ALLin™ Red Taq Mastermix and 2X ALLin™ Taq Mastermix are available.

See next pages.

BENEFITS

- Engineered Taq combined with advanced buffer - a synergy providing advantages over classical Taq Polymerases
- Higher yields under standard and fast cycling
- Increased success in amplification of longer templates (6 kb)
- Robust amplification of GC/AT rich templates
- 5X ALLin™ PCR Buffer contains optimal Mg²⁺ and dNTPs

PERFORMANCE



highQu ALLin™ Taq DNA Polymerase (above) shows better yields and higher sensitivity compared to competitor Taq DNA Polymerase (below).

PCR of a 1.2 kb fragment of 60% GC GAPDH, from human genomic DNA, in a 3 fold dilution from left to right. Starting from 200 ng of DNA up to 0.7 pg in the 7th dilution.



PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.
- Do not use fast cycling for multiplexing.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 50 μl reaction:

Rev. & For. Primers	0.1-0.4 μM final each (≤2 μl of 10 μM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
5X ALLin™ PCR Buffer	10 μl
Water (PCR Water, WAT0110)	to 49 μl
ALLin™ Taq DNA Polymerase, 5 u/μl	0.25 - 1 μl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C - 15 sec
Extension	40 cycles: 72°C - 1-90 sec (15 sec/kb)

- ✓ Store probes for short time on ice, for long at -20°C.

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PCM0201	200 r of 50 µl	5 x 1 ml - ALLin™ Red Taq Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading.
PCM0205	1000 r of 50 µl	25 x 1 ml - ALLin™ Red Taq Mastermix, 2X 25 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- Routine PCR up to 6 kb for a direct gel loading
- Amplification of complex (GC/AT rich) templates
- Colony PCR
- Fast PCR
- TA cloning

PRODUCT DETAILS

highQu ALLin™ Taq DNA Polymerase is the versatile engineered enzyme which in combination with the optimized ALLin™ buffer provides higher success rates in demanding PCR applications like amplification of complex templates, crude sample PCR and fast cycling.

ALLin™ Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, 4.5×10^4 (a number of correct nucleotides incorporated before the first error) and produces A-tailed products suitable for ligating into TA cloning vectors.

BENEFITS

- Engineered Taq combined with advanced buffer - a synergy providing advantages over classical Taq Polymerases
- Premixed with red dye and density reagents for direct loading on the gels after the PCR
- Higher yields under standard and fast cycling
- Increased success in amplification of longer templates (6 kb)
- Robust amplification of GC/AT rich templates

The convenience of ALLin™ Taq DNA Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.

ALLin™ Red Taq Mastermix, 2X is premixed with red dye and density reagents for direct loading on the gels after the PCR. In a 2% agarose TAE gel the dye migrates with ~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments. The mastermix is even supplied with PCR water.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.
- ALLin™ Red Taq Mastermix, 2X is premixed with red dye and density reagents for direct loading on the gels. In a 2% agarose TAE gel the dye migrates with ~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5 - 500 ng
PCR Water	to 25 µl
ALLin™ Red Taq Mastermix, 2X	25 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument set like: 	
Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C - 15 sec
Extension	40 cycles: 72°C - 1-90 sec (15 sec/kb)

- ✓ Load probes on the agarose gel. The red loading dye is included in the mastermix.
- ✓ Store probes for short time on ice, for long at -20°C.

ALLin™ Taq Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PCM0101	200 r of 50 µl	5 x 1 ml - ALLin™ Taq Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers.
PCM0105	1000 r of 50 µl	25 x 1 ml - ALLin™ Taq Mastermix, 2X 25 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- Routine PCR up to 6 kb
- Amplification of complex (GC/AT rich) templates
- Colony PCR
- Fast PCR
- TA cloning

BENEFITS

- Engineered Taq combined with advanced buffer - a synergy providing advantages over classical Taq Polymerases
- Higher yields under standard and fast cycling
- Increased success in amplification of longer templates (6 kb)
- Robust amplification of GC/AT rich templates

PRODUCT DETAILS

highQu ALLin™ Taq DNA Polymerase is the versatile engineered enzyme which in combination with the optimized ALLin™ buffer provides higher success rates in demanding PCR applications like amplification of complex templates, crude sample PCR and fast cycling.

The convenience of ALLin™ Taq DNA Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.

The mastermix is even supplied with PCR water.

ALLin™ Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, 4.5×10^4 (a number of correct nucleotides incorporated before the first error) and produces A-tailed products suitable for ligating into TA cloning vectors.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
PCR Water	to 25 µl
ALLin™ Taq Mastermix, 2X	25 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument set like: 	
Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C - 15 sec
Extension	40 cycles: 72°C - 1-90 sec (15 sec/kb)

- ✓ Store probes for short time on ice, for long at -20°C.

Taq DNA Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PCE0201	1500 u	1500 u - Taq DNA Polymerase, 5 u/μl 4 x 2 ml - 10X PCR Buffer 2 x 2 ml - 50 mM MgCl ₂	Enzyme in storage buffer. 10X PCR Buffer contains enhancers and stabilizers, but no dNTPs and no Mg ²⁺ .
PCE0202	3000 u	2 x 1500 u - Taq DNA Polymerase, 5 u/μl 8 x 2 ml - 10X PCR Buffer 4 x 2 ml - 50 mM MgCl ₂	

Storage: In the dark at -20°C.

APPLICATIONS

- Routine PCR up to 5 kb
- RT-PCR, Colony PCR
- TA cloning, library construction
- Genotyping, screening

PRODUCT DETAILS

highQu Taq DNA Polymerase is the classical enzyme for routine PCR applications providing high amplification yields of 3-5 kb targets under various conditions. Taq DNA Polymerase is purified from a recombinant *E. coli* strain carrying the Taq DNA polymerase gene. Taq DNA polymerase is thermostable 5' → 3' DNA polymerase. It lacks 3' → 5' exonuclease (proofreading) activity and has low 5' → 3' exonuclease activity. Polymerase exhibits deoxynucleotidyl transferase activity resulting in A-overhang at the 3'-ends of PCR products and allowing for TA cloning. The PCR accuracy of Taq DNA Polymerase is 4.5 x 10⁴ (a number of nucleotides incorporated before the first error occurs).

BENEFITS

- High yields in routine PCR, good performance in fast PCR
- Guaranteed successful DNA preparation for TA cloning
- Robust on complex templates

RECOMMENDATIONS

The supplied reaction buffer contains no dNTPs and the 50 mM MgCl₂ is provided separately what allows for magnesium optimization upon the need. dNTPs in mixes or sets can be purchased separately (page 43)

- Typical concentration of each dNTP in the reaction is 0.2 – 0.25 mM. Higher concentration increase yields, however Mg²⁺-ions bind to dNTPs, therefore, both components shall be present in coordinated concentrations. Too high dNTPs and magnesium concentrations reduce PCR fidelity.
- Mix well each dNTP and magnesium solution.
- Use final 3 mM MgCl₂ and 0.25 mM each dNTP concentrations for routine PCR.

Starting dNTP Mix conc.	Vol. of dNTP mix in 50 μl r.	Final Mg ²⁺ conc. in r.	Vol. of 50 mM MgCl ₂ in 50 μl rxn to achieve desired conc.
10 mM	1.25 μl	2 mM	2 μl
25 mM	0.5 μl	3 mM	3 μl

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time. Taq DNA Polymerase's speed is ~ 2000 nucleotides/min, so 15-90 seconds of extension can be provided per cycle, depending on amplicon size.
- Start annealing with 55°C and perform gradient by increasing temperature in 2°C up to 65°C to choose the best. Calculate primer annealing temperature using software.

- ✓ Prepare a 50 μl reaction:

Rev. & For. Primers	0.1-0.5 μM final (1-2 μl of 10 μM each)
cDNA Template or gDNA Template	< 100 ng or 5 - 500 ng
10X PCR Buffer	5 μl
dNTP Mix (NUM0201)	0.25 mM final (1.25 μl of 10 mM dNTP mix)
50 mM Mg Cl ₂	5 μl
Water (PCR Water, WAT0110)	to 49 μl
Taq DNA Polymerase, 5 u/μl	0.25 - 1 μl

- ✓ Mix gently, avoid bubbles. Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 60 sec
Denaturation	30-40 cycles: 95°C - 15 sec
Annealing	30-40 cycles: 55-65°C - 15 sec
Extension	30-40 cycles: 72 °C - 15-90 sec
Final Extension	1 cycle: 72°C - 5 min (for TA cloning)

- ✓ Store probes for short time on ice, for long at -20°C.

IN VITRO RESEARCH USE ONLY

ALLin™ Hot Start Taq Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HSE0101	500 u	2x 250 u - ALLin™ Hot Start Taq Polymerase, 5 u/μl 4 x 1 ml - 5X ALLin™ PCR Buffer	Enzyme in storage buffer. 1X ALLin™ PCR Buffer contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers.
HSE0105	2500 u	10 x 250 u - ALLin™ Hot Start Taq Polymerase, 5 u/μl 20 x 1 ml - 5X ALLin™ PCR Buffer	

Storage: In the dark at -20°C.

APPLICATIONS

- Sensitive hot-start PCR up to 6 kb
- Low copy target detection
- Amplification of complex (GC/AT rich) templates
- Fast PCR
- TA cloning
- Multiplex hot-start PCR
- Colony PCR

PRODUCT DETAILS

highQu ALLin™ Hot Start Taq Polymerase is the superior sensitive hot-start enzyme. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.

In combination with the optimized ALLin™ buffer enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling. ALLin™ Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, and produces A-tailed products suitable for ligating into TA cloning vectors. For the maximum convenience use 2X ALLin™ Hot Start Taq Mastermixes. *See next pages.*

BENEFITS

- Small molecular inhibition hot-start technology combined with advanced buffer - advantages over classical hot-start Taq
- Outperforming sensitivity & specificity - low copy target detection
- Higher yields under standard and fast cycling
- Increased success in amplification of longer templates (6 kb)
- Robust amplification of GC rich templates
- 5X ALLin™ PCR Buffer contains optimal Mg²⁺ and dNTPs

PERFORMANCE



highQu ALLin™ Hot Start Taq DNA Polymerase shows better yields and higher sensitivity compared to competitors.

PCR of a 0.4 kb fragment, from human genomic DNA, under fast cycling conditions. Starting template 100 ng with further 10 fold dilutions.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 50 μl reaction:

Rev. & For. Primers	0.1-0.4 μM final each (≤2 μl of 10 μM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
5X ALLin™ PCR Buffer	10 μl
Water (PCR Water, WAT0110)	to 49 μl
ALLin™ Hot Start Taq DNA Polymerase, 5 u/μl	0.25 - 1 μl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

- ✓ Store probes for short time on ice, for long at -20°C.

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HSM0301	200 r of 50 µl	5 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading.
HSM0305	1000 r of 50 µl	25 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X 25 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- Hot-start PCR up to 6 kb for a direct gel loading
- Crude sample and colony PCR
- Low copy target detection
- Amplification of complex (GC/AT rich) templates
- Fast PCR
- TA cloning
- Multiplex hot-start PCR

PRODUCT DETAILS

highQu ALLin™ Hot Start Taq DNA Polymerase is the superior sensitive enzyme. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.

In combination with the optimized ALLin™ buffer enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling.

ALLin™ Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, and produces A-tailed products suitable for ligating into TA cloning vectors.

BENEFITS

- Outperforming sensitivity & specificity - low copy number target detection and no background
- Higher yields under standard and fast cycling
- Increased sensitivity and success in amplification of longer templates (6 kb), robust amplification of GC rich templates
- Premixed with the red dye and density reagents for direct loading on the gels after the PCR

The convenience of ALLin™ Hot Start Taq DNA Polymerase is maximized by the use of 2X Mastermixes providing the additional advantage of reduced pipetting and minimized errors.

ALLin™ HS Red Taq Mastermix, 2X is premixed with the red dye and density reagents for direct loading on the gels after the PCR. In a 2% agarose TAE gel the dye migrates with ~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.

The mastermix is even supplied with PCR water.

ALLin™ HS Red Taq Mastermix, 2X is also a key component in highQu SampleIN™ Direct PCR Kit (DPK0101/5), ensuring outstanding PCR results with crude samples.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.
- ALLin™ HS Red Taq Mastermix, 2X is premixed with red dye and density reagents for direct loading on the gels. In a 2% agarose TAE gel the dye migrates with ~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
PCR Water	to 25 µl
ALLin™ HS Red Taq Mastermix, 2X	25 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

- ✓ Load probes on the agarose gel. The red loading dye is included in the mastermix.
- ✓ Store probes for short time on ice, for long at -20°C.

ALLin™ Hot Start Taq Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HSM0201	200 r of 50 µl	5 x 1 ml - ALLin™ Hot Start Taq Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers.
HSM0205	1000 r of 50 µl	25 x 1 ml - ALLin™ Hot Start Taq Mastermix, 2X 25 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- Sensitive hot-start PCR up to 6 kb
- Low copy target detection
- Amplification of complex (GC/AT rich) templates
- Fast PCR
- TA cloning
- Multiplex hot-start PCR
- Colony PCR

BENEFITS

- Small molecular inhibition hot-start technology combined with advanced buffer - advantages over classical hot-start Taq
- Outperforming sensitivity & specificity - low copy number target detection and no background
- Higher yields under standard and fast cycling
- Increased sensitivity and success in amplification of longer templates (6 kb)
- Robust amplification under difficult conditions - GC rich templates

PRODUCT DETAILS

highQu ALLin™ Hot Start Taq DNA Polymerase is the superior sensitive enzyme. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.

In combination with the optimized ALLin™ buffer enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling.

ALLin™ Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, and produces A-tailed products suitable for ligating into TA cloning vectors.

The convenience of ALLin™ Hot Start Taq DNA Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors. The mastermix is even supplied with PCR water.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

- ✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or	< 100 ng or
gDNA Template	5-500 ng
PCR Water	to 25 µl
ALLin™ Hot Start Taq Mastermix, 2X	25 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C – 15 sec
Extension	40 cycles: 72°C – 1-90 sec (15 sec/kb)

- ✓ Store probes for short time on ice, for long at -20°C.

IN VITRO RESEARCH USE ONLY

ALLin™ RPH Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLE0101	250 u	250 u - ALLin™ RPH Polymerase, 5 u/μl 2 x 1 ml - 5X ALLin™ RPH Buffer	Enzyme in storage buffer. 1X ALLin™ RPH Buffer contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers.
HLE0105	1250 u	5 x 250 u - ALLin™ RPH Polymerase, 5 u/μl 10 x 1 ml - 5X ALLin™ RPH Buffer	

Storage: In the dark at -20°C.

APPLICATIONS

- Amplification of difficult & complex (GC/AT rich) templates
- Long PCR (up to 35 kb) with higher fidelity
- Colony & crude sample PCR
- Multiplex PCR
- TA cloning

PRODUCT DETAILS

highQu ALLin™ RPH Polymerase (Robust, Proofreading, Hot-start Polymerase) is the versatile engineered enzyme combining best polymerase properties for excellence in most demanding PCR applications, like low copy detection, long or high fidelity PCR, amplification of complex templates, crude sample PCR and multiplexing.

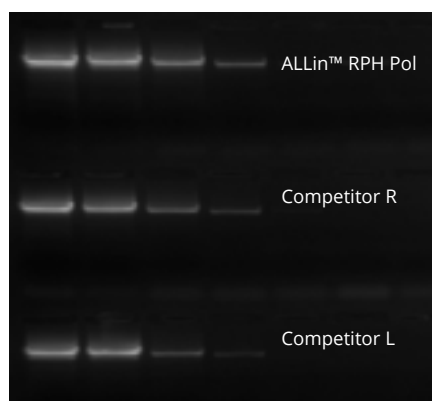
ALLin™ RPH Polymerase has 5 times higher fidelity than Taq DNA Polymerase and produces A-tailed products suitable for ligating into TA cloning vectors.

For the maximum convenience the 2X ALLin™ RPH Mastermix is available. See next page.

BENEFITS

- RPH – Robust, Proofreading, Hot-start Polymerase
- Low-copy number target detection ensured by small molecular inhibitor hot-start
- Long PCR (up to 35 kb), higher-fidelity (5X higher than Taq) – ensured by proofreading activity
- High yields under standard and fast cycling
- Robust - GC or AT rich templates, crude sample PCR
- 5X ALLin™ PCR Buffer contains optimal Mg²⁺ and dNTPs

PERFORMANCE



ALLin™ RPH Polymerase ensures highest sensitivity amplification of 25 kb target from lowest amounts of human genomic DNA. The starting template concentration is 200 ng of human genomic DNA with 2x further dilutions. 25kb fragment of the p53 gene was amplified

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension for amplicons of < 5 kb.
- Use 40 - 60 sec/kb extension for amplicons of 5 - 35 kb.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 50 μl reaction:

Rev. & For. Primers	0.1-0.4 μM final each (≤2 μl of 10 μM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
5X ALLin™ RPH Buffer	10 μl
Water (PCR Water, WAT0110)	to 49 μl
ALLin™ RPH Polymerase, 5 u/μl	0.25 - 1 μl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Place into the instrument set like: 	
Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 55-65°C - 15 sec
Extension	25-35 cycles: 72°C - 10 min
<ul style="list-style-type: none"> ✓ Store probes for short time on ice, for long at -20°C. 	

ALLin™ RPH Mastermix, 2X

bulk quantities available

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLM0101	200 r of 50 µl	5 x 1 ml - ALLin™ RPH Mastermix, 2X 5 x 1 ml - PCR Water	1X mastermix contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers.
HLM0105	1000 r of 50 µl	25 x 1 ml - ALLin™ RPH Mastermix, 2X 25 x 1 ml - PCR Water	

Storage: In the dark at -20°C.

APPLICATIONS

- Amplification of difficult & complex (GC/AT rich) templates
- Long PCR (up to 35 kb) with higher fidelity
- Colony & crude sample PCR
- Multiplex PCR
- TA cloning

BENEFITS

- RPH – Robust, Proofreading, Hot-start Polymerase
- Low-copy number target detection ensured by small molecular inhibitor hot-start
- Long PCR (up to 35 kb), higher-fidelity (5X higher than Taq) – ensured by proofreading activity
- High yields under standard and fast cycling
- Robust - GC or AT rich templates, crude sample PCR

PRODUCT DETAILS

highQu ALLin™ RPH Polymerase (Robust, Proofreading, Hot-start Polymerase) is the versatile engineered enzyme combining best polymerase properties for excellence in most demanding PCR applications, like low copy detection, long or high fidelity PCR, amplification of complex templates, crude sample PCR and multiplexing.

ALLin™ RPH Polymerase has 5 times higher fidelity than Taq DNA Polymerase and produces A-tailed products suitable for ligating into TA cloning vectors.

The convenience of ALLin™ RPH Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.

The mastermix is even supplied with PCR water.

PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time:
Use 15 sec/kb extension for amplicons of < 5 kb.
- Use 40-60 sec/kb extension for amplicons of 5-35 kb.
- Use 90 sec extension for multiplexing. Do not use fast cycling for multiplexing.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤2 µl of 10 µM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
PCR Water	to 25 µl
ALLin™ RPH Mastermix, 2X	25 µl

- ✓ Mix gently, avoid bubbles.

- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C – 1-2 min
Denaturation	25-40 cycles: 95°C – 15 sec
Annealing	25-40 cycles: 55-65°C – 15 sec
Extension	25-40 cycles: 72°C – 10 min)

- ✓ Store probes for short time on ice, for long at -20°C.

ALLin™ HiFi DNA Polymerase

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLE0201	200 u	200 u - ALLin™ HiFi DNA Polymerase, 2 u/μl 3 x 1 ml - 5X ALLin™ HiFi Buffer	Enzyme in storage buffer. 1X ALLin™ HiFi Buffer contains 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers.
HLE0205	1000 u	5 x 200 u - ALLin™ HiFi DNA Polymerase, 2 u/μl 15 x 1 ml - 5X ALLin™ HiFi Buffer	

Storage: In the dark at -20°C.

APPLICATIONS

- High-fidelity PCR up to 10 kb
- Long PCR up to 10 kb
- Amplification of complex (GC/AT rich) templates
- Fast high-fidelity PCR
- Blunt cloning
- Crude sample & colony PCR with high fidelity

PRODUCT DETAILS

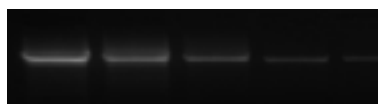
highQu ALLin™ HiFi DNA Polymerase is the outperforming high-fidelity enzyme derived from Pfu polymerase by introducing several point mutations. The robust engineered enzyme in combination with the optimized ALLin™ buffer provides higher fidelity (50X higher than Taq), better performance, increased success in demanding applications like amplification of complex or longer templates, crude sample PCR and fast cycling.

ALLin™ HiFi DNA Polymerase produces blunt-ended products suitable for ligating into blunt vectors. For end-polishing and phosphorylation of DNA ends before blunt-cloning use the HighEnd™ Repair Kit (HER0101).

BENEFITS

- Engineered proofreading enzyme and advanced buffer
- 50 x higher fidelity than classic Taq
- Increased sensitivity, high yield under standard and fast cycling
- Increased success in PCR of longer templates (10 kb)
- Robust on GC/AT rich templates, crude samples
- 5X ALLin™ PCR Buffer contains optimal Mg²⁺ and dNTPs

PERFORMANCE



highQu ALLin™ HiFi DNA Polymerase (above) shows better yields and higher sensitivity compared to P enzyme from competitor N (below).

PCR of a 1 kb fragment of 60% GC GAPDH, from human genomic DNA.

The template is diluted 2 fold over 8 orders of magnitude, starting from 100 ng.



PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- The longer the amplicon, the longer the extension time: 30 sec/kb.
- Run an annealing temperature gradient from 55°C to 65°C to choose the best specificity conditions.

- ✓ Prepare a 50 μl reaction:

Rev. & For. Primers	0.1-0.4 μM final each (≤2 μl of 10 μM)
cDNA Template or gDNA Template	< 100 ng or 5-500 ng
5X ALLin™ HiFi Buffer	10 μl
Water (PCR Water, WAT0110)	to 49 μl
ALLin™ HiFi DNA Polymerase, 2 u/μl	0.5 μl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 55-65°C - 15 sec
Extension	25-35 cycles: 72°C - 30 sec (30 sec/kb)

- ✓ Store probes for short time on ice, for long at -20°C.

IN VITRO RESEARCH USE ONLY

SampleIN™ Direct PCR Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
DPK0101	80 r of 50 µl	1.6 ml - DPK Lysis Buffer, 5X 0.8 ml - DPK Protease Buffer, 10X 2 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X	DPK Lysis Buffer, 5X contains all components required for an efficient lysis of mammalian tissue samples. DPK Protease Buffer, 10X contains proteases to eliminate sample proteins. 1X ALLin™ HS Red Taq Mastermix contains hot-start enzyme, 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading
DPK0105	400 r of 50 µl	5 x 1.6 ml - DPK Lysis Buffer, 5X 5 x 0.8 ml - DPK Protease Buffer, 10X 10 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X	
Storage:		In the dark at -20°C.	ALLin™ HS Red Taq Mastermix can be ordered separately: HSM0301 / HSM0305.

APPLICATIONS

- Fast direct PCR without template purification
- Mouse genotyping and knockout analysis
- Direct PCR from mouse tail or ear, mammalian tissues (including FFPE), hair follicle, buccal swabs and blood (including EDTA or FTA samples), plants

PRODUCT DETAILS

SampleIN™ Direct PCR Kit is a premium tool for a fast direct PCR eliminating the need of tedious template purification. The kit is excellent for direct PCR from mouse tail or ear, mammalian tissues, hair follicle, buccal swabs and blood. Rapid 15 min DNA extraction using DPK Lysis and Protease Buffers in a single tube generates PCR template extract which is further amplified under fast cycling conditions with a hot-start Taq master mix that includes red dye for direct gel loading. In a 2% agarose TAE gel the red dye migrates with ~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments. The ALLin™ HS Red Taq Mastermix includes a hot start Taq DNA Polymerase what ensures high yield, specific, low background amplification. Mix components allow for a fast PCR cycling and increase success when working with complex templates or multiplexing. Generated A-tailed PCR products are suitable for ligating into TA cloning vectors, sequencing and other applications.

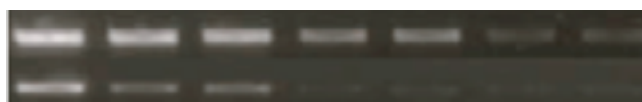
BENEFITS

- Ready-to load PCR in 50 minutes without template purification
- Single-tube 15 min DNA extraction combined with fast hot-start PCR
- Red dye in the PCR master mix for direct gel loading
- High yields under standard or fast cycling conditions
- Success with GC/AT rich templates

SAMPLE GUIDELINES

Sample (fresh or frozen)	Amount	Extraction vol.
Mouse tail	2 mm or 3-5 mg	100 µl
Mouse ear	2 mm ² or 3-5 mg	100 µl
Mammalian tissue	5 mg	100 µl
FFPE Tissue	2 mm ² of 10 µm section	100 µl
Blood (fresh/EDTA)	2 µl	100 µl
Blood Guthrie cards	2 mm ²	100 µl
Blood FTA/FTA Elute cards	2 mm ²	100 µl
Hair follicle	2 follicles	100 µl
Buccal swab	1 swab	300 µl

Sample amounts can be slightly increased for better yields, but too much material may cause inefficient lysis and PCR inhibition.



SampleIN™ Direct PCR Kit (upper) gives higher PCR yields from different dilutions of mouse tail sample extracts compared to competitors' kits (lower).

I. SAMPLE DNA EXTRACTION PROTOCOL

- Take typical measures to prevent contamination, keep your bench clean, wear gloves, and use sterile tubes.
- Thaw DPK Buffers at room temperature. Mix well before use.
- Prepare a 100 µl extraction reaction in a sterile vial (use 3x larger volumes of all reagents for buccal swab):

Sample amount	as above in SAMPLE GUIDELINES
DPK Lysis Buffer, 5X	20 µl
DPK Protease Buffer, 10X	10 µl
PCR Water (not supplied)	70 µl
✓ Mix gently, avoid bubbles. Place into the instrument set like:	
Lysis	75°C - 5 min. Vortex twice during lysis.
Protease inactivation	95°C - 10 min
- Add 900 µl of PCR Water. Centrifuge 1 min to pellet cell debris.
- Remove supernatant into the sterile tube.
- Store it at -20°C for several months or use immediately for PCR.

IN VITRO RESEARCH USE ONLY

II. PCR PROTOCOL

- ✓ Prepare a 50 µl PCR reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤ 2 µl of 10 µM)
Template	1-5 µl of extraction supernatant
PCR Water	to 25 µl
ALLin™ HS Red Taq Mastermix, 2X	25 µl
✓ Mix gently, avoid bubbles. Place into the instrument set like:	
Initial denaturation	1 cycle: 95°C - 2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C - 15 sec
Extension	40 cycles: 72°C - 15 sec/kb (90 sec for multiplex)

- ✓ Load probes on the gel. The red loading dye is included in PCR mix.
- ✓ Store probes for short time on ice, for long at -20°C.

The use of this product in certain countries for certain applications may be covered by patents and may require a license

highQu product range for reverse transcription and RT PCR provides convenient kits and master mixes to ensure safety of RNA samples and to shorten time to results due to the reduced number of pipeting steps and due to accelerated reactions.

Our thermostable reverse transcriptase allows for high yields of full lengths transcripts of up to 12-15 kb and for cDNA synthesis from complex templates.

Order a sample today at www.highqu.com/samplerequest

Bulk orders are welcome at order@highqu.com

RT PCR & Reverse Transcription Enzymes & Kits



RT PCR & Reverse Transcription Selection

	1Step RT PCR Kit	HighScriber™ Reverse Transcriptase Mix	qScriber™ cDNA Synthesis Kit
	<i>page 45</i>	<i>page 46</i>	<i>page 47</i>
<i>Short description</i>	One-step RT PCR in one tube	cDNA synthesis of long transcripts at elevated temperature	Unbiased cDNA synthesis for qPCR
<i>Enhanced cDNA synthesis</i>	●	●	●
<i>GC rich and complex templates</i>	●	●	●
<i>Full-length cDNA transcripts up to 15 kb</i>		●	
<i>High sensitivity</i>	●	●	●
<i>One-step RT-PCR</i>	●		
<i>Two-step RT-PCR</i>		●	●
<i>Two-step RT-qPCR</i>		●	●
<i>RNA protection from RNases</i>	●	●	●

1Step RT PCR Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RTK0201	100 r of 50 µl	2 x 1.25 ml - 1Step RT PCR Mastermix, 2X 2 x 0.125 ml - RT2 Mix, 20X 3 x 1 ml - PCR Water	1X mastermix contains hot-start Taq DNA Polymerase, 0.25 mM dNTPs, 3 mM MgCl ₂ , enhancers, stabilizers. RT2 Mix is a 20X concentrated blend of reverse transcriptase and RNase inhibitor

Storage: In the dark at -20°C.

APPLICATIONS

- One step RT-PCR
- RT-PCR of complex GC/AT rich templates
- Fast RT-PCR
- TA cloning

PRODUCT DETAILS

highQu 1Step RT PCR Kit combines the blend of Reverse Transcriptase and RNase Inhibitor for efficient reverse transcription and the PCR Mastermix for subsequent amplification of cDNA in the same tube. RT2 Mix, 20X is a blend of the engineered MMuLV (stable at 40-55°C allowing for high yields of long transcripts) with an efficient Ribonuclease Inhibitor protecting the template RNA from RNases. The resulting cDNA is then amplified by the 1Step RT PCR Mastermix, 2X. The PCR mastermix contains our proprietary Hot Start Taq DNA Polymerase. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.

BENEFITS

- Easy to use combination of the RT mix with the RT-PCR mastermix - reverse transcription and PCR in one tube
- RT Mix contains RNase inhibitor and thermostable reverse transcriptase (up to 55°C) allowing for high cDNA yields
- RT PCR Mastermix allows for sensitive low copy number targets detection due to proprietary hot-start
- High yields under standard and fast cycling conditions and on GC/AT rich templates

In combination with the optimized buffer the enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling. Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase and produces A-tailed products suitable for ligating into TA cloning vectors.

For the maximum convenience the Kit includes even the PCR Water to set up the reaction, so the only thing you need to take care is the high quality RNA template.

PROTOCOL

- RNA is extremely sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation and to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control, no RT2 Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Perform cDNA synthesis 10 min at 45°C, 20 min for >1 kb, increase temperature to 55°C for complex templates.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension for amplicons of <3 kb.
- Use 40-60 sec/kb extension for amplicons of 5-10 kb.
- Run an annealing temperature gradient from 58°C to 65°C to choose the best specificity conditions.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.2-0.4 µM final (≤2 µl of 10 µM each)
Total RNA	1 pg to 1 µg or
mRNA	> 0.01 pg
PCR Water	to 22.5 µl
1Step RT PCR Mastermix, 2X	25 µl
RT2 Mix, 20X	2.5 µl

- ✓ Mix gently, avoid bubbles.

- ✓ Place into the instrument set like:

Reverse transcription	1 cycle: 45 - 55°C - 10 to 20 min
Initial denaturation	1 cycle: 95°C - 2 min
Denaturation	40 cycles: 95°C - 10 sec
Annealing	40 cycles: 60-65°C - 10 sec
Extension	40 cycles: 72°C - 30-60 sec (15 sec/kb)

- ✓ Store probes for short time on ice, for long at -20°C.

HighScriber™ Reverse Transcriptase Mix, 20X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RTM0301	10000 u / 50 r	2 x 25 µl - HighScriber™ Reverse Transcriptase Mix, 20X 2x 0.2 ml - 5X ALLin™ HighScriber Reaction Buffer	Enzyme Mix contains HighScriber™ RT at 200 u/µl concentration, Ribonuclease Inhibitor and glycerol. Reaction Buffer includes magnesium, DTT and dNTPs.
RTM0305	50000 u / 250 r	10 x 25 µl - HighScriber™ Reverse Transcriptase Mix, 20X 10 x 0.2 ml - 5X ALLin™ HighScriber Reaction Buffer	

Storage: In the dark at -20°C.

APPLICATIONS

- cDNA synthesis of up to 15 kb long transcripts
- Template generation for RT-PCR & RT-qPCR
- cDNA synthesis from complex templates

PRODUCT DETAILS

The HighScriber™ Reverse Transcriptase Mix is a premium tool for the high efficiency reverse transcription of up to 12-15 kb long cDNA. Mix includes HighScriber™ RT at 200 u/µl concentration and Ribonuclease Inhibitor for safe cDNA synthesis. HighScriber™ Reverse Transcriptase allows for high detection sensitivity from 1 pg of total RNA. The wide reaction temperature range (38°C - 55°C) ensures efficient cDNA synthesis from complex or GC rich templates. The enzyme uses ssRNA or ssDNA as a template, possesses no detectable Ribonuclease H activity specific to RNA in RNA-DNA hybrids. A highly reduced Ribonuclease H activity allows for transcription of full lengths long transcripts. HighScriber™ RT can be used for RACE as it has terminal transferase activity - adds cytosines to 3' ends of cDNA.

BENEFITS

- Thermostable Reverse Transcriptase blended with RNase Inhibitor for an efficient cDNA synthesis
- High yields of full lengths transcripts up to 12-15 kb
- cDNA synthesis from complex templates at up to 55°C
- High sensitivity detection from 1 pg of total RNA template

PRODUCT SPECIFICATIONS

- Optimal activity at 45-50°C
- Temperature range 38-55°C
- Inactivation at 85°C for 10 min

The Ribonuclease inhibitor premixed with the RT ensures RNA protection from ribonuclease degradation. Supplied 5X ALLin™ HighScriber Buffer includes everything you need for the cDNA synthesis reaction: it contains MgCl₂, dNTPs, enhancers, stabilizers. The only things to add is the template RNA and primer.

One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly (rA) oligo (dT)₁₈ as template.

PROTOCOL

- RNA is extremely sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Check the integrity of RNA prior to cDNA synthesis in denaturing agarose gel.
- Include positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- For best results, optimize the template and primer amount.
- Do not exceed the recommended amount of the enzyme.
- Perform reaction for 30-50 min, for short transcripts 15-30 min are sufficient.
- Choose optimal reaction temperature in a range of 42-55°C.

Do not add Ribonuclease Inhibitor and dNTPs, as they are already included in supplied Mix and buffer.

- ✓ Prepare a 20 µl reaction:

5X ALLin™ HighScriber Reaction Buffer	4 µl (includes dNTPs!)
Oligo dT primer or Random primer or Specific primer	0.5 µg or 0.2 µg or 15-20 pmol
Total RNA or Poly-A mRNA	1 pg to 5 µg or 1 pg to 0.5 µg
Water (PCR Water, WAT0110)	to 19 µl
<ul style="list-style-type: none"> ✓ Mix gently, avoid bubbles. ✓ Heat 5 min at 65°C, spin, place on ice for 1 min. 	
<ul style="list-style-type: none"> ✓ Incubate 2 min at 42°C for Oligo dT and for Specific primer or 10 min at 25°C for Random primer to anneal. 	
<ul style="list-style-type: none"> ✓ Add 1 µl HighScriber™ RTase Mix, 20X and mix well. ✓ Incubate 30-50 min at 50°C to synthesize cDNA. ✓ Inactivate at 85°C for 10 min. ✓ Store reactions at -20°C or on ice for an immediate use. ✓ Use 2-5 µl of this reaction mix per 50 µl PCR reaction. ✓ Use 1-2 µl of this reaction mix per 20 µl qPCR reaction. 	

IN VITRO RESEARCH USE ONLY

qScriber™ cDNA Synthesis Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RTK0101	25 r of 20 µl	25 µl - qScriber™ Enzyme Blend, 20X 100 µl - 5X qScriber™ Reaction Mix 1 ml - PCR Water	Enzyme Blend Storage buffer contains Tris, 50% glycerol and other components.
RTK0104	100 r of 20 µl	4 x 25 µl - qScriber™ Enzyme Blend, 20X 4 x 100 µl - 5X qScriber™ Reaction Mix 2 x 1 ml - PCR Water	5X qScriber™ Reaction Mix contains dNTPs, MgCl ₂ , anchored oligo(dT), random hexamers and other components.
<i>Storage:</i>		<i>In the dark at -20°C.</i>	

APPLICATIONS

- cDNA template generation for qPCR or PCR
- Unbiased, efficient cDNA synthesis
- Detection of low target amounts
- cDNA synthesis from complex templates

PRODUCT DETAILS

The qScriber™ cDNA Synthesis Kit is a highly efficient and simple-to-use system for cDNA synthesis eliminating the need for tedious reaction optimization. The qScriber™ Enzyme Blend ensures high sensitivity detection from low copy number targets. The highly active and thermostable HighScriber™ Reverse Transcriptase blended with RNase Inhibitor allows for an efficient cDNA synthesis and reaction safety. The wide reaction temperature range (38°C - 55°C) ensures efficient transcription from GC rich templates.

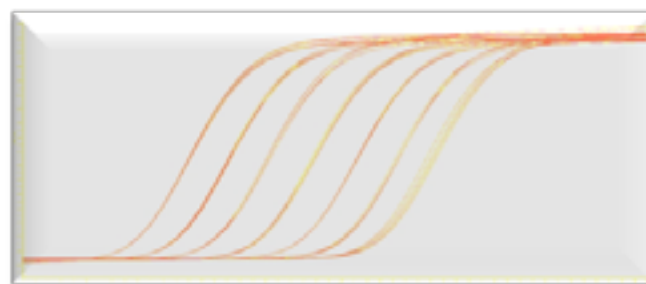
The 5X qScriber™ Reaction Mix includes optimal concentrations of magnesium and dNTPs and a combination of anchored oligo (dT) and random hexamers for unbiased representation of mRNA ends. The kit is an optimal choice for generating high quality cDNA from viral RNA, miRNA or other targets for qPCR or for PCR.

BENEFITS

- Thermostable HighScriber™ Reverse Transcriptase blended with Ribonuclease Inhibitor for efficient cDNA synthesis
- Optimized reaction mix with oligo (dT) and random primers for unbiased representation of mRNA ends
- cDNA synthesis from complex templates at up to 55°C
- High sensitivity detection from 1 pg of total RNA template

PERFORMANCE

qScriber™ cDNA Synthesis Kit provides excellent results within the very broad range of the total RNA amount used.



4 pg, 40 pg, 400 pg, 4 ng, 40 ng, 400 ng and 4 µg of mouse total RNA were used for cDNA synthesis under the standard qScriber™ cDNA Synthesis Kit protocol conditions. An aliquot from each reaction was taken for subsequent qPCR with ORA™ qPCR Green Mix to amplify a 70 bp fragment of the mouse RN18S gene. All reactions independently from the initial amount of RNA were 100% efficient.

PROTOCOL

- RNA is sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include positive and negative controls in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not add any other components into the reaction with exception of the template and the supplied reagents.
- The recommended reaction temperature is 42-50°C. For GC rich templates, the temperature can be increased up to 55°C.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

5X qScriber™ Reaction Mix	4 µl
qScriber™ Enzyme Blend, 20X	1 µl
Total RNA	1 pg to 5 µg
PCR Water	up to 20 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Incubate 30 min at 42-50°C to synthesize cDNA.
- ✓ Inactivate the enzyme at 85°C for 10 min.
- ✓ Store reactions at -20°C or on ice for an immediate use.
- ✓ Use 2-4 µl of this reaction mix per 20 µl qPCR reaction.

What else do you need for PCR?

Uracil DNA Glycosylase helps to eliminate crossover contamination in your PCR or qPCR. Try our UDGIN™ PCR Cleaner Mix, 20X, a convenient product to be added to all highQu PCR and qPCR mixes, if needed.

Our PCRbeam™ Fast PCR Detection Kit is a unique tool for fast detection of gene-specific amplification products without the need to run an agarose gel.

Order a sample today at www.highqu.com/samplerrequest

Bulk orders are welcome at order@highqu.com

PCR-related Reagents & Kits



PCR-related Reagents Overview

	UDGin™ PCR Cleaner Mix, 20X	PCRbeam™ Fast PCR Detection Kit	dNTP Sets & Mixes	PCR Water
	<i>page 51</i>	<i>page 52</i>	<i>page 53</i>	<i>page 54</i>
<i>Applications</i>	Prevention of carry-over contamination in qPCR and PCR	Detection of PCR products. Alternative to gels and qPCR	PCR/qPCR, DNA/cDNA synthesis	Molecular biology applications including PCR and qPCR

UDGin™ PCR Cleaner Mix, 20X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
UDG0101	500 r of 20 µl	0.5 ml - UDGin™ PCR Cleaner Mix, 20X	20X master mix for PCR carry-over contamination prevention, contains optimized concentration of dUTP, Uracil DNA Glycosylase (UDG), and stabilizers.

Storage: In the dark at -20°C.

APPLICATIONS

- Efficient prevention of carry-over contamination in qPCR and PCR

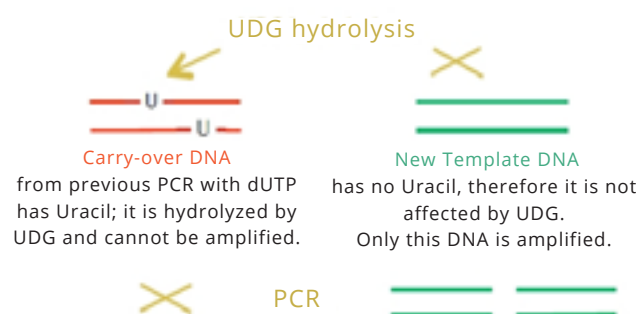
PRODUCT DETAILS

UDGin™ PCR Cleaner Mix is an efficient tool for carry-over contamination prevention in qPCR or PCR. It is optimized for the use in conjunction with highQu qPCR or PCR products. UDGin™ PCR Cleaner Mix, is a ready to use 20X mix of optimally combined Uracil-DNA Glycosylase (UDG) and dUTP in a buffer with stabilizers. 1 µl of UDGin™ PCR Cleaner Mix added into each 20 µl PCR reaction prevents amplification of DNA carried over from previous PCR performed with dUTP. During 10 minutes incubation before the PCR start, UDG hydrolyzes the N-glycosilic bond between uracil and sugar leaving apyrimidinic sites in uracil containing DNA which is then cleaved by heat during the first PCR cycle, thus only the newly added template is amplified. UDG is inactivated during the initial PCR denaturation step and does not destroy newly synthesized dUTP containing DNAs. PCR products obtained when UDGin™ PCR Cleaner Mix was used contain uracil, therefore they will be destroyed again by UDG before the next PCR start.

BENEFITS

- Time saving ready-to-use mix with UDG and dUTP
- Compatible with all highQu qPCR and PCR master mixes

PCR CARRY-OVER CONTAMINATION PREVENTION



If you use UDGin™ PCR Cleaner Mix in every PCR, only the newly added template DNA will be amplified. All PCR products will contain uracil. Therefore, even if you carry them over into the next PCR they will not be amplified, as the uracil containing DNA will be destroyed during UDG treatment before each PCR. Only new added DNA containing no uracil will serve as PCR template.

Reference: Longo, M.C., et al., Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions, Gene 93, 125-8, 1990.

PROTOCOL

- Follow the protocol of ORA™ qPCR Mix or ALLin™ PCR Enzyme or Mastermix that you use. Only three additional steps are generally required for preventing carry-over contamination in PCR using UDGin™ PCR Cleaner Mix:
1. Before adding water into PCR reactions, add 1µl of UDGin™ PCR Cleaner Mix, 20X into each 20 µl reaction. Use less water accordingly.
 2. Set your qPCR/PCR instrument to perform at the beginning one cycle of 37°C incubation for 10 min.
 3. After incubation always perform one cycle of longer initial denaturation (and UDG inactivation) for 5 minutes at 95°C.
- Store PCR reactions on ice.
 - Before subsequent applications, consider that your PCR products contain uracil. This has no influence on electrophoresis and sequencing, but might affect the cleavage with certain restriction enzymes (check enzyme performance on U containing sites). When cloning, use only ung- bacterial hosts for transformations.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

Reverse Primers	100 - 400 nM final c.
Forward Primers	100 - 400 nM final c.
cDNA Template or gDNA Template	< 100ng or < 1 µg
UDGin™ PCR Cleaner Mix, 20X	1 µl (to final 1X conc.)
PCR Water	to 10 µl
ORA™ Green ROX L Mix, 2X	10 µl

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument (SYBR® Green or FAM channel) set like:

UDG treatment	1 cycle: 37°C - 10 min (uracil-containing DNA hydrolysis)
Initial denaturation	1 cycle: 95°C - 5 min (DNA denaturation, UDG inactivation , Hot-start Polymerase activation)
Denaturation	40 cycles: 95°C - 5 sec
Annealing/ Extension	40 cycles: 60-65°C - 20-30 sec

- ✓ Store probes for short time on ice, for long at -20°C.

The use of this product in certain countries for certain applications may be covered by patents and may require a license

PCRbeam™ Fast PCR Detection Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PDK0101	50 tests	50 - PCRbeam™ Membrane Strip 10 ml - PCRbeam™ Detection Buffer	PCRbeam™ Membrane Strips are coated with biotin-ligand (for test band) and anti-FITC antibody in gold conjugate (for test band) and anti-FITC antibody in gold conjugate PCRbeam™ Detection Buffer is Tris-buffered saline.

Storage: In the dark at -20°C.

APPLICATIONS

- Low throughput PCR, LAMP, RPA based tests
- Sensitive detection of specific amplification products
- Fast and 20x more sensitive alternative to EtBr stained gels
- Economical alternative to qPCR-based detection

PRODUCT DETAILS

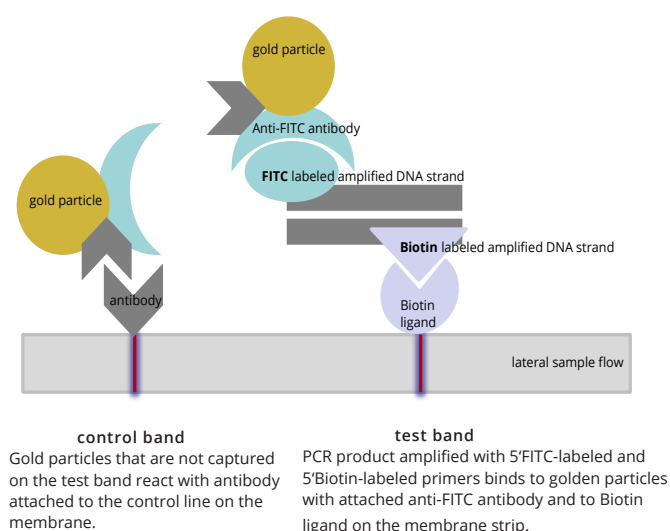
highQu PCRbeam™ Fast PCR Detection Kit is a convenient tool for fast detection of gene-specific amplification products obtained by PCR, LAMP or RPA. The detection is based on immunological reaction driven by Biotin and FITC (fluorescein isothiocyanate), thus the amplified DNA shall include Biotin and FITC labels. The PCR amplification has to be performed with one primer labeled with FITC at 5'-end and one primer labeled with Biotin at 5'-end. Alternatively the use of one of the labeled primers can be replaced by gene-specific FITC or Biotin labeled probe. Kit includes PCRbeam™ Membrane Strips that are coated with biotin-ligand on the test band and an anti-rabbit antibody on the control band. The bottom part of the strip which is used for sample application contains an anti-FITC antibody attached to gold particles. PCRbeam™ Detection Buffer is Tris-buffered saline enabling the detection.

The PCRbeam™ Fast PCR Detection Kit can be applied for established tests or home-brew assays as a fast and sensitive yes/no detection method. The detection sensitivity is up to 100 fold higher than the one achievable with ethidium bromide stained gels what provides an environment friendly save and economical alternative to the use of mutagen stains. For establishing sensitive PCR-based tests before PCRbeam™ detection we recommend the use of hot-start PCR enzymes or master mixes, like highQu ALLin™ Hot Start Taq Mastermix or ALLin™ Hot Start Taq DNA Polymerase.

BENEFITS

- Sensitive detection of PCR, LAMP, RPA gene-specific products
- No gel loading after PCR, no ethidium bromide handling
- Saved costs compared to qPCR-based detection methods
- Fast and easy procedure with little hands on time

PRINCIPLE



The membrane strip is soaked for 10 minutes into the vial with the detection buffer mixed with PCR product. The lateral sample flow driven by gold particles moves the solution up the strip. FITC labeled DNA strand binds with the anti-FITC antibody on the gold particle and Biotin labeled DNA strand is caught by Biotin ligand attached to the test band. As both DNA strands remain hybridized at room temperature, the test band builds an aggregate that develops red-blue color. Excess gold particles that were not caught by FITC move up the strip and the anti-FITC antibody binds to the anti-rabbit antibody to develop the red-blue colored control band. If there is no PCR product in the reaction, then only the control band will be visible. If there is a specific product, the test band will be colored as well.

NOTES

- Optimize and perform PCR with one primer labeled with FITC at 5'-end and one primer labeled with Biotin at 5'-end.
- Apply the PCRbeam™ Fast PCR Detection Kit only for established PCR assays, as a yes/no detection tool.
- Up to 5 pg DNA can be detected using PCRbeam™ Kit.
- Before starting the detection procedure warm the PCRbeam™ Membrane Strips and PCRbeam™ Detection Buffer at room temperature for 5 minutes.
- Avoid carrying over of the mineral oil when pipetting the PCR products for detection. Oil interferes with detection as it affects the lateral flow of the sample.

PROTOCOL

- Pipet 100 µl of the PCRbeam™ Detection Buffer into the plate or into the marked empty PCR vials.
- Add 5-10 µl of PCR product into each vial with detection buffer. Mix by gentle pipetting. Use up to 20 µl of the PCR mixture in case low yield is suspected.
- Insert the PCRbeam™ Membrane Strip into each vial so that the indicated spot for sample is soaked in the liquid.
- Incubate at room temperature for 2-10 min until the control band (if positive, the test band as well) gets red-blue color.
- Interpret the results immediately as yes (2 bands: control and test) or no (1 control band), independently on the intensity of the color of the bands.

25 mM and 10 mM dNTP Mixes & 100 mM dNTP Set

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
dNTP Mixes:			
NUM0101	1 ml	1 ml - 25 mM dNTP Mix	Aqueous solution of equal concentration of 25 mM each of 4 dNTPs, pH 7.0
NUM0201	1 ml	1 ml - 10 mM dNTP Mix	Aqueous solution of equal concentration of 10 mM each of 4 dNTPs, pH 7.0
dNTP Sets:			
NUS0101	4 x 0.25 ml	0.25 ml -100 mM dATP	Aqueous solution of 100 mM dATP, pH 7.0
		0.25 ml - 100 mM dCTP	Aqueous solution of 100 mM dCTP, pH 7.0
		0.25 ml - 100 mM dGTP	Aqueous solution of 100 mM dGTP, pH 7.0
		0.25 ml - 100 mM dTTP	Aqueous solution of 100 mM dTTP, pH 7.0
Storage:	In the dark at -20°C.		

APPLICATIONS

All molecular biology applications including dNTPs, like:

- cDNA synthesis
- Standard PCR, Long and high-fidelity PCR
- qPCR
- Sequencing

BENEFITS

- Highest quality, >99% HPLC pure dNTPs for high & reproducible yields
- Pure from DNA contamination and from PCR inhibitors
- Highly stable remain pure after weeks at room temperature, after 30 freezing thawing cycles and during the 40 PCR cycles
- Available in ready-to use mixes and sets for maximized flexibility

PRODUCT DETAILS

highQu dNTP sets and mixes meet all highest industry standards and allow for unrivaled performance of your PCR and other DNA synthesis reactions.

Produced under the stringent quality monitoring conditions, they guaranty reproducible results. More than 99% HPLC purity eliminates inhibitions of PCR and allows for increased yields with higher dNTP concentrations.

Exceptional stability eliminates dNTP usability concerns related to short term ambient temperature shipments, room temperature storage or PCR exceeding 40 cycles.

PREPARATION OF DNTP MIXES FROM A SET

- Highly concentrated solutions require thorough mixing before the use.
- The optimal dNTP mix shall have equal concentrations of all 4 dNTPs.
- To prepare from a set of 4 dNTPs mixes of common concentrations, follow the guidelines below:

Use same volume of each from four 100 mM dNTP solutions:	PCR Water	Resulting 1 ml Mix concentration:
20 µl	920 µl	2 mM dNTP
25 µl	900 µl	2.5 mM dNTP
100 µl	600 µl	10 mM dNTP
250 µl	-	25 mM dNTP

IN VITRO RESEARCH USE ONLY

PROTOCOL RECOMMENDATIONS FOR STANDARD PCR

- Typical concentration of each dNTP in the reaction is 0.2 - 0.25 mM. Higher concentration increase yields, however Mg^{2+} ions bind to dNTPs, therefore, both components shall be present in coordinated concentrations. Too high dNTPs and magnesium concentrations reduce PCR fidelity.
- Mix well each dNTP and magnesium solution, to avoid concentration fluctuations.
- Use final 3 mM $MgCl_2$ with 0.25 mM each dNTP concentration for routine PCR.

Starting dNTP Mix conc.	Vol. of dNTP mix in 50 µl r.	Final Mg^{2+} conc. in r.	Vol. of 50 mM $MgCl_2$ in 50 µl rxn to achieve desired conc.
10 mM	1.25 µl	2 mM	2 µl
25 mM	0.5 µl	3 mM	3 µl

PCR Water

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
WAT0110	10 x 1 ml	10 x 1 ml - PCR Water	PCR-grade, nuclease free water
<i>Storage: In the dark at -20°C. To minimize the effects of the contamination during the use it is not recommended to store water at room temperature.</i>			

PRODUCT DETAILS

highQu PCR Water is a supplementary high quality reagent for all demanding applications. It saves time being on your bench and guaranties purity of reactions and inhibition-free performance of PCR reagents.

highQu PCR Water is a deionized, membrane filtered water continuously tested in ultrasensitive qPCR and PCR applications, in amplification of long targets and highly specific detection of few copies of templates.

APPLICATIONS

- All molecular biology applications

BENEFITS

- Pure PCR-grade, nuclease free water for excellence in molecular biology applications
- Same PCR Water is supplied with most of highQu products for maximum convenience and guaranteed performance
- Tested in most demanding PCR and qPCR applications

PROTOCOL

- Use PCR Water in all PCR applications like described in typical protocols.

IN VITRO RESEARCH USE ONLY

Cloning Kits



Cloning Kits Overview

	Rally™ Rapid Ligation Kit	HighEnd™ Repair Kit
	page 57	page 58
Applications	Fast vector-insert ligation, adaptor or linker ligation, linear DNA self-circularization	Preparation of PCR products, shared or nebulized DNA, restriction-digested DNA, cDNA for blunt-end ligation. Conversion of 5'- and/or 3'-protruding ends to 5'-phosphorylated blunt-ended ones

Rally™ Rapid Ligation Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RLK0101	40 r of 20 µl	40 µl – Rally™ T4 DNA Ligase, 1 r/µl 1.5 ml - 2X Rally™ Buffer	Storage buffer contains Tris, 50% glycerol and other components. 2X Rally™ Buffer contains DTT, ATP, PEG 6000 and other components.
RLK0105	200 r of 20 µl	5 x 40 µl – Rally™ T4 DNA Ligase, 1 r/µl 5 x 1.5 ml - 2X Rally™ Buffer	

Storage: In the dark at -20°C.

APPLICATIONS

- Cloning
- Vector-insert ligation
- Adaptor or linker ligation
- Linear DNA self-circularization

PRODUCT DETAILS

Rally™ Rapid Ligation Kit is a premium tool designed for fast and highly efficient ligation reactions, cloning or adaptor/linker joining applications. The Kit contains Rally™ T4 DNA Ligase specially formulated to perform faster; and a 2X buffer which includes PEG to accelerate joining of DNA ends.

The combination of both components allows for an efficient and fast ligation reaction of both blunt and cohesive DNA termini eliminating the need of hours or overnight incubations.

BENEFITS

- 5 minutes fast high efficiency ligation
- Universal for both blunt or cohesive-end ligations
- Premium reagents - reproducible results

PRODUCT SPECIFICATIONS

- Optimum activity at room temperature, around 25°C
- Inactivation at 65°C for 15 min

Rally™ T4 DNA Ligase, same as the classical enzyme catalyzes the formation of a phosphodiester bond between the terminal 5' phosphate and 3' hydroxyl groups of DNA or RNA. It joins both blunt and cohesive ends and repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

PROTOCOL

- Check the integrity and the concentration of the DNA prior the ligation.
- Include ligation positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use. If precipitation in the buffer appears, warm it to dissolve.
- Use an insert : vector molar ratio around 3 : 1 to 6 : 1, optimize it if possible. Too much insert causes ligation of multiple inserts, too little reduces ligation efficiency.
- The Rally™ Buffer includes PEG which has almost no effect on transformation efficiency of chemically-competent cells, but reduces the transformation efficiency of electro-competent cells. Therefore, for electrotransformation PEG has to be removed from the mixture or highly diluted.
- Use only high efficiency competent cells for cloning. Check the transformation efficiency of the competent cells by transforming 0.1 ng of supercoiled vector DNA. Getting 100 colonies in this case means you have 1mln transformants/1 µg supercoiled DNA. Take into account that the ligated mixture gives normally at least 10 times less transformants compared to the same amount of supercoiled DNA.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 20 µl reaction:

2X Rally™ Buffer	10 µl
Linear dephosphorylated vector DNA	~100 ng (20 – 200 ng range)
<i>100 ng of linear pUC vectors (~2.7 kb) have ~0.1 pmol ends</i> <i>100 ng of linear pBR322 vectors (~4.4 kb) have ~0.07 pmol ends</i>	
Insert DNA (phosphorylated)	~200 ng (up to 3-6 X more pmol ends than vector)
<i>200 ng of 1 kb linear DNA has ~0.6 pmol ends</i> <i>100 ng of 0.5 kb linear DNA has ~0.6 pmol ends</i> <i>40 ng of 0.2 kb linear DNA has ~0.6 pmol ends</i>	
DNase-free water (PCR Water, WAT0110)	to 19 µl
Rally™ T4 DNA Ligase, 1 r/µl	1 µl maximum

- ✓ Mix well, incubate for 5-10 min at 25°C (room temperature).
- ✓ Use directly 1-5 µl of the mixture to transform 50 µl of chemically-competent cells.
- ✓ For electrotransformation PEG has to be removed from the mixture or diluted as follows:

Option 1: re-purify the ligation mixture using PCR clean-up spin column kit, elute in 20 µl of water or TE and transform 1-2 µl of the eluate into 50 µl of electro-competent cells.

Option 2: immediately after ligation dilute the mixture 10X by adding 180 µl of water or TE and transform 2-5 µl of the diluted mix into 100 µl of electro-competent cells.

HighEnd™ Repair Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HER0101	40 r of 25 µl	40 µl – HighEnd™ Repair Blend, 1 r/µl 1.5 ml - 10X HighEnd™ Buffer 0.5 ml - 1 mM dNTP Mix	Enzyme blend storage buffer contains Tris, 50% glycerol and other components. 10X HighEnd™ Buffer contains Tris, NaCl, MgCl ₂ , DTT, and other components.
HER0105	200 r of 25 µl	5 x 40 µl – HighEnd™ Repair Blend, 1 r/µl 2 x 1.5 ml - 10X HighEnd™ Buffer 3 x 0.5 ml - 1 mM dNTP Mix	dNTPs serve as building blocks for filling-in reaction and as phosphate donors for phosphorylation.

Storage: In the dark at -20°C.

APPLICATIONS

- Preparation of PCR products, shared or nebulized DNA, restriction-digested DNA, cDNA for blunt-end ligation
- Conversion of 5'- and/or 3'-protruding ends to 5'-phosphorylated blunt-ended ones

PRODUCT DETAILS

HighEnd™ Repair Kit is a premium tool designed for rapid and highly efficient DNA end-repair before the ligation reactions. PCR products, shared or nebulized DNA, restriction-digested DNA and cDNA can be blunted/phosphorylated in a couple of minutes and are ready for an efficient blunt-end ligation and cloning.

The Kit includes HighEnd™ Repair Blend – an optimized mix of T4 DNA Polymerase and T4 Polynucleotide Kinase. The 5'→3' polymerase and 3'→5' exonuclease activities of T4 DNA Polymerase form the blunt-ended DNA. T4 Polynucleotide Kinase phosphorylates 5' DNA ends. The resulting DNA is a high quality blunt-ended substrate for T4 DNA Ligase.

BENEFITS

- Fast and simple blunting and phosphorylation of DNA at once
- Universal - preparing any kind of DNA for blunt-end ligation
- Premium reagents - reproducible results

PRODUCT SPECIFICATION

- Optimum activity at room temperature, around 25°C
- Inactivation at 75°C for 20 min

Up to 1-5 microgram of the linear DNA can be blunted and phosphorylated in one 20 min reaction. After the thermal inactivation the reaction mixture can be used for blunt-end ligations.

HighEnd™ Repair Kit is an ideal choice for preparing for ligations the PCR products obtained with high fidelity polymerases like ALLin™ HiFi DNA Polymerase (HLE0201).

PROTOCOL

- Check the integrity and the concentration of the DNA prior the reaction.
- Always repurify PCR products before end-repair.
- Thaw and keep reagents on ice. Mix all components well before use.
- The optimal DNA amount in the reaction depends on the lengths of the DNA fragment. For example 1 µg of 1 kb linear DNA has ~3 pmol ends, but 1 µg of 100 bp linear DNA has 10X more substrate for blunting/phosphorylation; i.e. even 30 pmol DNA ends. Therefore, the shorter is the DNA fragment, the less of it shall be used in micrograms for end-repair or for later ligation reaction.
- For high DNA amounts upscale the reaction accordingly. For example 5 µg of short 100 bp fragment can be end-repaired in 100 µl reaction using 2-5 µl of HighEnd™ Repair Blend.

IN VITRO RESEARCH USE ONLY

- ✓ Prepare a 25 µl reaction:

Linear DNA in TE buffer or water	up to 1 µg (up to 30 pmol ends)
----------------------------------	---------------------------------

1 µg of 1 kb linear DNA has ~3 pmol ends

1 µg of 0.5 kb linear DNA has ~6 pmol ends

1 µg of 0.1 kb linear DNA has ~30 pmol ends

10X HighEnd™ Buffer	2.5 µl
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1 mM dNTP Mix	2.5 µl
---------------	--------

DNase-free water (PCR Water, WAT0110)	to 24 µl
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HighEnd™ Repair Blend, 1 r/µl	1 µl (max 2 µl)
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- ✓ Mix well; incubate for 20 - 30 min at 25°C.
- ✓ Inactivate enzymes at 75°C for 20 min and keep cooled in case the ligation is performed immediately or keep frozen in case the ligation is performed later.
- ✓ Alternatively, re-purify the DNA using PCR clean-up spin column kit, elute in 25 µl of water or TE and keep frozen.
- ✓ For subsequent ligation and cloning follow the recommendations for Rally™ Rapid Ligation Kit (RLK0101).

Ladders & Stains for DNA & Protein Electrophoresis



Selection of DNA Electrophoresis Ladders

DNA LADDER LADDER BANDS IN BP AND IN KB, ALL REFERENCE BANDS IN BOLD

Take5™ 50 bp	50 - 100 - 150 - 200 - 250 - 300 - 350 - 400 -- 500 - 600 - 700 - 800 - 900 - 1 - 1,2 -- 1,5
Take5™ 100 bp	----- 100 ----- 200 ----- 300 ----- 400 -- 500 - 600 - 700 - 800 - 900 - 1 ----- 1,5 ----- 3
Take5™ 1kb	--- 100 ----- 200 ----- 300 ----- 400 -- 500 - 600 - 700 - 800 - 900 - 1 ----- 1,5 - 2 - 2,5 - 3 - 4 - 5 - 6 - 8 - 10
Take5™ HR	----- 250 ----- 500 ----- 750 ----- 1 ----- 1,5 - 2 - 2,5 - 3 - 4 - 5 - 6 - 8 - 10 - 25

Selection of DNA Electrophoresis Stains

FEATURES OF NUCLEIC ACID STAINS	StainIN™ RED Nucleic Acid Stain <i>page 62</i>	StainIN™ GREEN Nucleic Acid Stain <i>page 63</i>
Fluorescence	Red	DNA green, RNA red
Excitation max.	540 nm	490 nm
Emission max.	630 nm	520 nm and 635 nm
In gel staining during agarose electrophoresis	Yes	Yes
Staining of PAGE during electrophoresis	Yes	Yes
Post-run staining	-	-
DNA detection sensitivity	0,3 - 0,6 ng	0,1 - 0,3 ng
UV detection	Yes	Yes
Blue light detection	-	Yes
Used as loading dye	-	-
Cloning compatible	Yes, when UV exposure is minimal	Yes, under Blue light
Filters to use	Ethidium Bromide	SYBR® Green

DNA Electrophoresis Ladders

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
DNL0102	200 appl.	2 x 0.5 ml - Take5™ 1 kb DNA Ladder 2 x 1 ml - Take5™ Loading Dye, 6X	Ready to load ladders contain highly purified PCR products combined with plasmid digests, they are supplied in 1x loading dye: 10 mM Tris-HCl (pH 8.0) 10 mM EDTA, glycerol and tracking dyes. 6X Take5™ Loading Dye includes 10 mM Tris-HCl (pH 8.0) 60 mM EDTA, glycerol and three electrophoresis tracking dyes (Xylene cyanol FF, Bromophenol blue, Orange G). <i>1 µl of 6X dye shall be used for 5 µl of DNA sample, mixed well and loaded.</i>
DNL0202	200 appl.	2 x 0.5 ml - Take5™ 100 bp DNA Ladder 2 x 1 ml - Take5™ Loading Dye, 6X	
DNL0302	200 appl.	2 x 0.5 ml - Take5™ 50 bp DNA Ladder 2 x 1 ml - Take5™ Loading Dye, 6X	
DNL0402	200 appl.	2 x 0.5 ml - Take5™ HR DNA Ladder 2 x 1 ml - Take5™ Loading Dye, 6X	

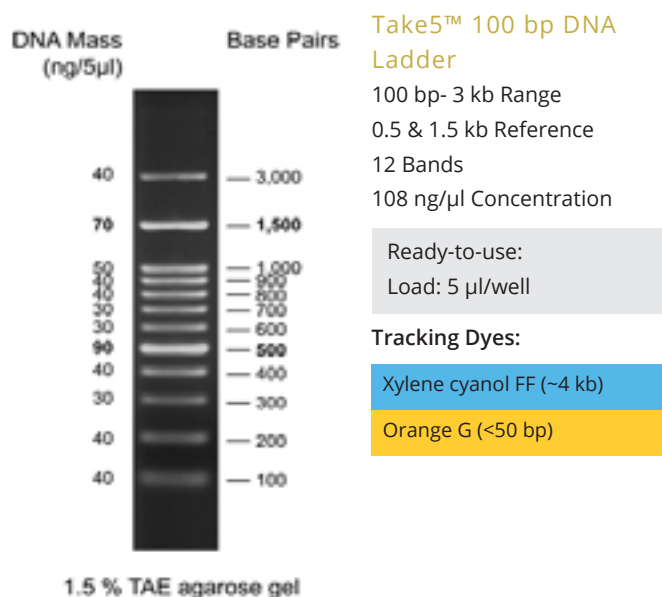
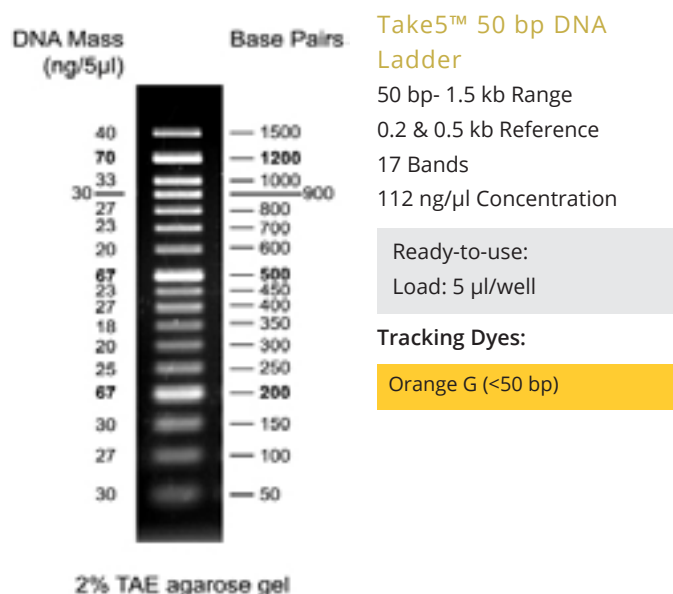
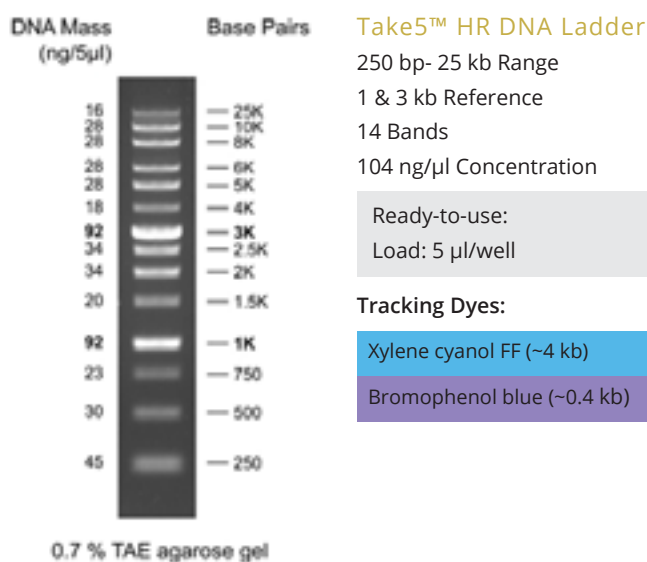
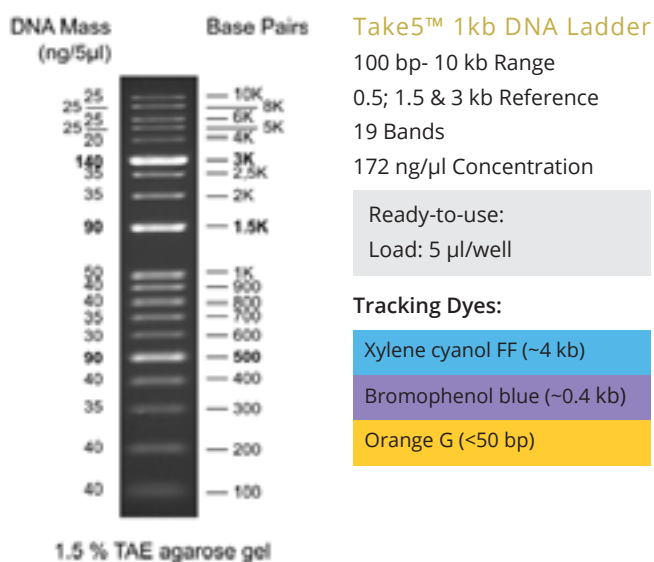
IN VITRO RESEARCH USE ONLY

APPLICATIONS

- DNA size determination and approximate DNA quantification on agarose gels

BENEFITS

- Room-temperature-stable, always ready to be used
- Sharp bands, bright reference bands, indicated DNA mass
- Take5™ ladders are supplied with loading dye for DNA samples



Stable: Room temperature - 6 months; at +4°C - 12 months; at -20°C - 24 months

StainIN™ RED Nucleic Acid Stain

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
NAS0101	1 ml	1ml - StainIN™ RED Nucleic Acid Stain	Red DNA and RNA stain in diluted DMSO, as 20000 X solution to be used at 1 X concentration in agarose or polyacrylamide gels and at 0,5 X concentration in electrophoresis buffers.
<i>Storage:</i>		<i>In the dark at +4°C.</i>	
<i>Disposal:</i>		<i>Used dye solutions or melted gels shall be run through filters and later disposed with plenty of water down the drain.</i>	

APPLICATIONS

- Staining of NA in agarose and polyacrylamide gels during electrophoresis for ssDNA, dsDNA and RNA visualization and gel documentation under the UV light

PRODUCT DETAILS

StainIN™ RED Nucleic Acid Stain is a significantly safer alternative to ethidium bromide. It is same easy to use, twice as sensitive and much more secure. At least twice as economical as competing products, this novel stain can be also used and disposed with less environmental and health concerns compared to ethidium bromide.

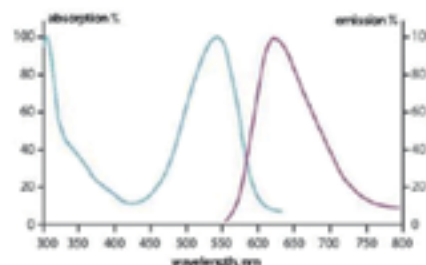
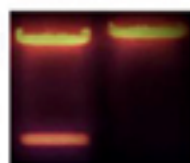
StainIN™ RED is a fluorescent dye that allows detection of >0,3 ng of DNA in both agarose and polyacrylamide gels. It binds to both ds DNA, ssDNA and RNA and emits red fluorescence detectable under the UV light and documented with same filters as ethidium bromide. For cloning applications, UV exposure shall be minimized.

Much smaller than ethidium bromide carcinogenicity of the dye has been proved by Ames-test. Mammalian cell mutagenicity tests, both mouse marrow erythrocyte micronucleus and spermatocyte chromosomal aberration tests gave negative mutagenicity results.

BENEFITS

- Much more safe alternative to ethidium bromide
- Highly sensitive NA detection - up to 2x more sensitive than EtBr
- Time saving – in gel stain, no post- run staining, no destaining

PERFORMANCE



Left image - agarose gel stained with StainIN™ RED Nucleic Acid Stain.

Right image - StainIN™ RED excitation maxima - 540 nm, emission - 630 nm.

PROTOCOL FOR AGAROSE GEL ELECTROPHORESIS

1. Wear gloves when working with all NA stains, buffers and gels.
2. Prepare the agarose gel solution like recommended by supplier.
3. Cool down the agarose after boiling to a hand-friendly temperature.
4. Add 5µl of StainIN™ RED solution per 100 ml of the gel right before casting the gel.
5. Mix the gel solution very gently to distribute the dye but not to produce air bubbles. Pour the gel, insert the combs.
6. Prepare the required volume of 1X TAE or 1X TBE buffer to be used in the electrophoresis tank.
7. Add 2,5 - 3 µl of StainIN™ RED solution per 100 ml of the 1X electrophoresis running buffer.
8. Add both gel and the buffer into the electrophoresis tank and run electrophoresis like usual.
9. Visualize nucleic acids under the UV light.
 - Destaining is not needed, but it might help to reduce the background; post-run staining is not recommended.
 - Use Ethidium bromide filters for gel photography.
 - After a few runs refresh the electrophoresis buffer in electrophoresis tank prepared as in step 7.
 - If you reuse the gel, add at least half a portion of the stain each time after boiling and cooling the gel solution down (like in step 4).

PROTOCOL FOR PAGE

1. Wear gloves when working with all NA stains, buffers and gels.
2. Prepare the native or denaturing PAA gel like recommended by supplier.
3. Add TEMED and APS and proceed to the next step immediately.
4. Add 5µl of StainIN™ RED solution per 100 ml of the gel right before casting the gel.
5. Mix the gel solution very gently to distribute the dye but not to produce air bubbles. Pour the gel, insert the combs.
6. Prepare the required volume of 1X TBE buffer to be used in the electrophoresis tank.
7. Add 2,5 - 3 µl of StainIN™ RED solution per 100 ml of 1X running buffer.
8. Add both gel and the buffer into the electrophoresis tank and run electrophoresis like usual.
9. Visualize nucleic acids under the UV light.
 - Destaining is not needed, post-run staining is not recommended.
 - Use Ethidium bromide filters for gel photography.
 - After a few runs refresh the electrophoresis buffer in electrophoresis tank prepared as in step 7.

IN VITRO RESEARCH USE ONLY

StainIN™ GREEN Nucleic Acid Stain

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
NAS0201	1 ml	1ml - StainIN™ GREEN Nucleic Acid Stain	Aqueous solution of green DNA and RNA stain, as 20000 X solution to be used at 1 X concentration in agarose or polyacrylamide gels and at 0,5 X concentration in electrophoresis buffers.
Storage:	In the dark at +4°C.		
Disposal:	Used dye solutions or melted gels shall be run through filters and later disposed with plenty of water down the drain.		

APPLICATIONS

- Staining of NA in agarose and polyacrylamide gels during electrophoresis for ssDNA, dsDNA and RNA visualization and gel documentation
- UV or Blue LED detection, excellent for cloning applications

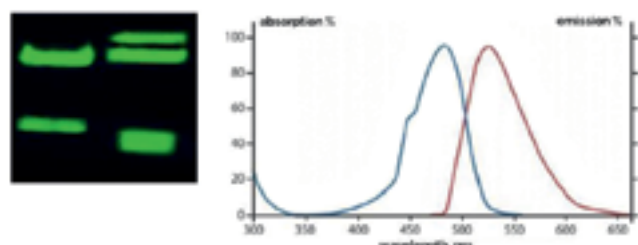
PRODUCT DETAILS

StainIN™ GREEN Nucleic Acid Stain is a significantly safer alternative to ethidium bromide. It is same easy to use, four times as sensitive and much more secure. Twice as economical as competing green dyes, this novel stain can be also used and disposed with less environmental and health concerns compared to ethidium bromide. It is a fluorescent dye that allows detection of >0,1 ng of DNA in both agarose and PAA gels. It binds to both dsDNA, ssDNA and RNA and emits green fluorescence when bound to DNA and red fluorescence when bound to RNA detectable under the UV or Blue light and documented with same filters like other green dyes. StainIN™ GREEN is ideal for DNA extraction from gels for cloning. Much smaller than ethidium bromide carcinogenicity of the dye has been proved by Ames-test. Mammalian cell mutagenicity tests, mouse marrow erythrocyte micronucleus and spermatocyte chromosomal aberration tests gave negative mutagenicity results.

BENEFITS

- Much more safe alternative to ethidium bromide, more economical alternative to competing green dyes
- Unique – two emission peaks, colors DNA in green, RNA in red
- Highly sensitive NA detection - up to 4x more sensitive than EtBr
- Time saving – in gel stain, no post- run staining, no destaining

PERFORMANCE



Left image - agarose gel stained with StainIN™ GREEN Nucleic Acid Stain. Right image - StainIN™ GREEN excitation maxima - 490 nm, emission maximas – bound to DNA - 520 nm; bound to RNA - 635 nm (not shown here).

PROTOCOL FOR AGAROSE GEL ELECTROPHORESIS

1. Wear gloves when working with all NA stains, buffers and gels.
2. Prepare the agarose gel solution like recommended by supplier.
3. Cool down the agarose after boiling to a hand-friendly temperature.
4. Add 5µl of StainIN™ GREEN solution per 100 ml of the gel right before casting the gel.
5. Mix the gel solution very gently to distribute the dye but not to produce air bubbles. Pour the gel, insert the combs.
6. Prepare the required volume of 1X TAE or 1X TBE buffer to be used in the electrophoresis tank.
7. Add 2,5 - 3 µl of StainIN™ GREEN solution per 100 ml of the 1X running buffer.
8. Add both gel and the buffer into the electrophoresis tank and run electrophoresis like usual.
9. Visualize nucleic acids under Blue light or UV (~500-650 nm).
 - Destaining is not needed, but it might help to reduce the background; post-run staining is not recommended.
 - Use only Blue light if you intend to clone the DNA.
 - Use SYBRGreen filters for gel photography.
 - After a few runs refresh the electrophoresis buffer in electrophoresis tank prepared as in step 7.
 - If you reuse the gel, add at least half a portion of the stain each time after boiling and cooling the gel solution down (like in step 4).

PROTOCOL FOR PAGE

1. Wear gloves when working with all NA stains, buffers and gels.
2. Prepare the native or denaturing PAA gel like recommended by supplier.
3. Add TEMED and APS and proceed to the next step immediately.
4. Add 5µl of StainIN™ GREEN solution per 100 ml of the gel right before casting the gel.
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8. Add both gel and the buffer into the electrophoresis tank and run electrophoresis like usual.
9. Visualize nucleic acids under Blue light or UV (~500-650 nm).
 - Destaining is not needed, post-run staining is not recommended.
 - Use SYBRGreen filters for gel photography.
 - After a few runs refresh the electrophoresis buffer in electrophoresis tank prepared as in step 7.

IN VITRO RESEARCH USE ONLY

Selection of Protein Electrophoresis Ladders

PROTEIN LADDER	COLORED LADDER BANDS IN KDA (TRIS-GLYCINE, 4-20% GRADIENT GEL)
Cozy™ Prestained	----- 11 -- 17 ----- 25 -- 35 -- 48 -- 63 -- 75 -- 100 -- 135 --- 180
CozyHi™ Prestained	5 --- 11 - 17 --20-- 25 -- 35 -- 48 -- 63 -- 75 -- 100 -- 135 --- 180 ---- 245
CozyXL™ Prestained	----- 11 -- 17 ----- 25 -- 35 -- 48 -- 63 -- 75 -- 100 --135 --- 180 ----- 245 --- 310

Protein Electrophoresis Ladders

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
PRL0102	200 appl.	2 x 0.5 ml - Cozy™ Prestained Protein Ladder	Ready to load ladders contain highly purified prestained proteins, supplied denatured in 20 mM Tris-Phosphate (pH 7.5 at 25°C), 2% SDS, 0.2 mM DTT, 3.6 M Urea, 15% (v/v) glycerol).
PRL0202	200 appl.	2 x 0.5 ml - CozyHi™ Prestained Protein Ladder	
PRL0302	200 appl.	2 x 0.5 ml - CozyXL™ Prestained Protein Ladder	

IN VITRO RESEARCH USE ONLY

APPLICATIONS

- Approximate protein molecular weight determination on denaturing gels and Western blots
- Monitoring of electrophoresis process and transfer efficiency

BENEFITS

- Room-temperature-stable, always ready to be used
- Sharp bands, bright colors

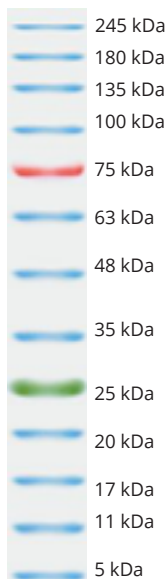
Cozy™

11 - 180 kDa Range
25 & 75 kDa References
10 Bands; 0.2-0.4 µg/µl each



CozyHi™

5 - 245 kDa Range
25 & 75 kDa References
13 Bands; 0.2-0.4 µg/µl each



CozyXL™

11 - 310 kDa Range
25 & 75 kDa References
12 Bands; 0.2-0.4 µg/µl each



Images taken after **Tris-Glycine 4-20% gel** electrophoresis show guidelines for approximate protein MW estimation. Given molecular weight of each protein is very approximate. It has been determined by calibrating it against unstained protein of same size. For precise sizing, such calibration shall be done exactly at conditions used.

PROTOCOLS

Ready-to-use prestained protein ladders:

- Thaw if needed, mix and load: 3- 5 µl/gel well for electrophoresis
- Load: 3- 5 µl/gel well for Western transfers
- For Western transfer, use 100 V, 90 minutes. To see high MW bands better, perform slower overnight transfer.
- Transfer Buffer: 25 mM Tris, 192 mM glycine, 20% methanol.

Stability:

- Room-temperature-stable for 2 weeks
- at +4°C for 3 months
- at -20°C for 12 months

PRODUCT USE LIMITATIONS

All products in this catalog have been developed, designed and are sold exclusively for research purposes and in vitro use only. These products have not been tested for use in diagnostics or drug development, nor are they suitable for administration to humans or animals.

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professionally simple

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