Check the product label for actual catalog number, lot and expiry date.

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 $MgCl_2$	Enzyme in storage buffer. 10X PCR Buffer contains enhancers and stabilizers, but no dNTPs and no Mg^{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

BENEFITS

- High yields in routine PCR and under fast cycling conditions
- Guarantied successful DNA preparation for TA cloning
- Robust on complex templates

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 10^4 (nucleotides incorporated before the error occurs) and it produces A-tailed products suitable for ligating into TA cloning vectors.

MAGNESIUM AND NUCLEOTIDE USE

The 50 mM $MgCl_2$ is provided in a separate tube what allows for magnesium optimization. The dNTPs in mixes or sets can be purchased separately.

- 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 dNTP and magnesium solutions, to avoid concentration fluctuations. Use final 3 mM $MgCl_2$ and 0.25 mM each dNTP concentrations for routine PCR.

Starting dNTP Mix conc.	Vol. of dNTP mix per 50 µl r.
10 mM	1.25 µl
25 mM	0.5 µl

Final Mg^{2+} conc. in r.	Vol. of 50 mM $MgCl_2$ per 50 µl rxn to achieve desired conc.
2 mM	2 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 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 (10 mM dNTP Mix NUM0201)	0.25 mM final (1.25 µl of 10 mM dNTP mix or 0.5 µl of 25 mM dNTP mix)
50 mM Mg Cl_2	3 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:

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

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