NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Plasmid DNA MiniPrep 96-Well Kit — Product Insert

Product #25900

Norgen's Plasmid DNA MiniPrep 96-Well Kit is designed for the rapid high throughput preparation of plasmid DNA from small batch cultures of *Escherichia coli*. The plasmid DNA is preferentially purified from other cellular components such as genomic DNA and RNA. The Plasmid DNA MiniPrep 96-Well Kit is able to purify plasmids up to 13,000 bp in size, and the typical purification yield is up to 20 µg from 1.5 mL of bacterial culture. Preparation time for a single 96-Well Plate is less than 90 minutes, and each kit contains sufficient materials for 96 preparations. The purified DNA is fully digestible with all restriction enzymes tested, and is completely compatible with manual or automated sequencing to achieve 95-100% accuracy.

Norgen's Purification Technology

Purification is based on 96-well column chromatography. The process for the isolation of plasmid DNA involves first pelleting 1.5 mL of an overnight culture of *E. coli* harbouring the plasmid of interest using centrifugation. The pellet is then resuspended in the provided Resuspension Buffer, which contains RNase A. Lysis Solution is then added to the sample in order to assist in the lysis of the bacterial cells. Next, Binding Solution is added to the sample which will neutralize the sample and cause precipitation of the proteins and genomic DNA that is present. The resulting suspension is spun down, and the clarified lysate containing the plasmid DNA is filtered through a 96-Well Filter Plate and then applied to a provided plate through centrifugation. Norgen's 96-Well Plate binds DNA in a manner that depends on ionic concentrations, thus the DNA will bind to the plate while most of the RNA, proteins and other contaminants will either flowthrough or be retained on top. The bound DNA is then washed twice using the provided Wash Solution in order to remove any remaining impurities, and the purified PCR product is eluted with the Elution Buffer.

Specifications

Kit Specifications	
Size of Plasmids Purified	Up to 13,000 bp
Average Yield from 1.5 mL of Culture	Up to 20 µg
Time to Complete 96 Purifications	90 minutes

Advantages

- High throughput purification of plasmid DNA – up to 96 samples
- Fast and easy processing using a rapid 96-well format
- High binding capacity – up to 25 µg
- High yield of plasmid DNA – up to 20 µg from 1.5 mL of culture

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers. The RNAse should be stored at -20°C upon arrival. The **Resuspension Buffer** should be stored at 4°C upon addition of RNAse enzyme.

Kit Components

Component	Product #25900 (96 preps)
Resuspension Buffer	24 mL
Lysis Solution	30 mL
Binding Solution	40 mL
Wash Solution	12 mL
Elution Buffer	20 mL
RNAse	1 vial
96-Well Deep Plate	1
96-Well Filter Plate	1
96-Well Plate	1
Adhesive Tape	6
96-Well Collection Plate	2
96-Well Elution Plate	1
Product Insert	1

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

The **Binding Solution** contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes.
- 96 – 100% ethanol
- Vortex
- Centrifuge with rotor for 96-well plate assembly, such as AllSpin Js-5.3 Rotor for Avanti® J-26xp centrifuge, Beckman Coulter or similar rotor that can hold the stack of the 96-well Plate and the 96-Well Collection Plate and that can reach the minimum speed of 4000 RPM (~4000xg)

Flow Chart

Procedure for Purifying Plasmid DNA using Norgen's Plasmid DNA MiniPrep 96-Well Kit

Pellet overnight bacterial culture containing plasmid of interest

Add Resuspension Buffer.
Add Lysis Solution and mix.
Add Binding Solution and mix.

SPIN

Transfer to 96-Well
Filter Plate

SPIN

Bind clarified lysate to
96-well plate

SPIN

Wash with Wash Solution.
Dry Spin.

SPIN

Elute DNA into Elution
Plate with Elution Buffer

Purified Plasmid DNA

Procedure

All centrifugation steps are performed at room temperature. The correct RPM can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\ (r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes prior to use:

- Ensure that all solutions, except the **Resuspension Buffer,** are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Add the entire amount of **RNAse** to the **Resuspension Buffer**. The label on the bottle has a box that can be checked to indicate that the RNAse has been added. The solution can be stored for up to 6 months at 4°C.
- Prepare a working concentration of **Wash Solution** by adding 48 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
- Bacterial cultures grown overnight at 37°C in LB medium are optimal for this procedure.
- For growing bacterial cultures in a **96-Well Deep Plate**: Aliquot 1.25 mL of LB medium with the appropriate antibiotic into each well of the 96-Well Deep Plate. Inoculate each well with a single bacterial colony. Cover the plate with the provided Adhesive Tape and use a needle to punch 2-3 small holes above each well to promote aeration (a Micropore tape can be used). Incubate the plate for 16-24 hours at 37°C with shaking at 250-300 RPM.

1. Lysate Preparation

i) Bacterial culture grown in tubes:

a. Transfer 1.5 mL of bacterial culture to a microcentrifuge tube and centrifuge for 30 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. Add 200 µL of **Resuspension Buffer** (containing **RNAse**; see Notes Prior to Use) to the cell pellet. Resuspend the cells by pipetting in and out, or by gentle vortexing. Incubate at room temperature for 5 minutes.

c. Add 250 µL of **Lysis Solution** to the cell suspension, cap the tube, and mix the contents by gently inverting the tube several times. Do not vortex as this will shear the genomic DNA. The suspension should become clear and viscous as the cells begin to lyse.

Continue mixing until the mixture becomes clear. If necessary, allow the solution to incubate at room temperature provided the total incubation time is no more than 5 minutes. This step is also critical for the denaturation of cellular proteins and genomic DNA.

d. Add 350 μL of **Binding Solution** and immediately mix by inverting the tube several times. The solution will become turbid as insoluble particles from denatured materials start to form.

e. Centrifuge for 5 minutes to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.

f. Transfer the supernatant in each tube into the designated well of the **96-Well Filter Plate**. Avoid or minimize the transfer of the insoluble pellicle.

g. Seal the 96-Well Filter Plate and centrifuge for 5 minutes at 4,000 RPM. **KEEP THE FILTRATE** to be used in the binding step.

Note: Ensure the entire lysate has passed through into the 96-Well Collection Plate by inspecting the 96-Well Filter Plate. If the entire wash volume has not passed, centrifuge for an additional 5 minutes.

iI) Bacterial culture grown in 96-Well Deep Plate:

a. Centrifuge the sealed 96-Well Deep Plate containing the grown culture for 5 minutes at 2,100 x g to pellet the cells. Remove the Adhesive Tape and pour off the supernatant carefully so as not to disturb or dislodge the cell pellets.

b. Add 200 μL of **Resuspension Buffer** (containing **RNAse**; see Notes Prior to Use) to the cell pellet in each well. Resuspend the cells by pipetting in and out, or by gentle vortexing. Incubate at room temperature for 5 minutes.

c. Add 250 μL of **Lysis Solution** to the cell suspension, seal the plate, and mix the contents by gently inverting several times. The suspension should become clear and viscous as the cells begin to lyse.

Continue mixing until the mixture becomes clear. If necessary, allow the solution to incubate at room temperature provided the total incubation time is no more than 5 minutes. This step is also critical for the denaturation of cellular proteins and genomic DNA.

h. Remove the seal and add 350 μL of **Binding Solution** and immediately seal the plate and mix by inverting several times. The solution will become turbid as insoluble particles from denatured materials start to form.

i. Centrifuge the plate for 5 minutes at 4,000 RPM to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.

j. Assemble a **96-Well Filter Plate** with a **96-Well Collection Plate**.

k. Transfer the supernatant in each well onto the corresponding well of the 96-Well Filter Plate. Avoid or minimize the transfer of the insoluble pellicle.

l. Seal the 96-Well Filter Plate and centrifuge for 5 minutes at 4,000 RPM. **KEEP THE FILTRATE** to be used in the binding step.

Note: Ensure the entire lysate has passed through into the 96-Well Collection Plate by inspecting the 96-Well Filter Plate. If the entire wash volume has not passed, centrifuge for an additional 5 minutes.

2. Binding DNA to 96-Well Plate

a. Place the **96-Well Plate** on top of the other **96-Well Collection Plate**.

Note: The user should ensure that the assembled 96-Well Plate and the 96-Well Collection Plate stack fits into the rotor without interfering with the centrifugation process.

b. Apply the filtrate produced from the lysate preparation step into each well of the 96-Well Plate. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 4 minutes.
c. Discard the flowthrough. Reassemble the 96-Well Plate and the 96-Well Collection Plate.

Note: Ensure that all of the lysate from each well has passed through into the 96-Well Collection Plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

3. DNA Wash

a. Apply 500 µL of **Wash Solution** to each well of the 96-Well Plate. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the 96-Well Collection Plate by inspecting the 96-Well Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Pat the bottom of the 96-Well Plate on an absorbent tissue to dry.
c. Reassemble the 96-Well Plate and the 96-Well Collection Plate. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 15 minutes in order to completely dry the 96-Well plate.
d. Pat the bottom of the 96-Well Plate on an absorbent tissue to dry.

4. DNA Elution

a. Stack the 96-Well Plate on top of the **96-Well Elution Plate**.
b. Add 100 µL of **Elution Buffer** to each well of the 96-Well Plate.
c. Centrifuge the assembly at 5,000 RPM (or a minimum of 4,000 RPM) for 3 minutes.

Note:
The entire elution volume will not be recovered, as up to 50 µL might be retained on each well. However this will not affect the recovered yield of DNA.

5. Storage of DNA

Use the provided adhesive tape to seal the 96-Well Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The 96 well Plate is clogged.	Cell debris may be clogging the 96-Well Plate	Ensure that the lysate has been spun down and then filtrated through the provided 96-Well Filter Plate before applying onto the 96-Well Plate
	The sample is too large	Do not exceed the recommended sample input volume of the initial overnight grown bacterial culture

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Plasmid did not propagate	Ensure that the appropriate growth medium, supplements and antibiotics were used for the host cell and plasmid of interest.
	Inoculum cell culture was old	Old bacterial cells are a poor source of plasmid DNA. Bacterial cell inoculum should be prepared from fresh single colonies, grown in a test-tube overnight and immediately used for inoculum preparation. Prolonged incubation or storage of culture in the fridge almost guarantees poor results.
	Insufficient lysis of cells	The **Lysis Solution** may have formed precipitates. Warm and mix gently before use.
	Cell resuspension was incomplete	Pelleted cells should be completely resuspended in the **Resuspension Buffer**. Do not add **Lysis Solution** until a homogeneous suspension is obtained.
	Elution Buffer was not placed directly over the column's membrane	It is important that the **Elution Buffer** be placed directly over the membrane inside each 96-Well Plate to ensure uniform passing of the buffer through the column. Do not pipette the **Elution Buffer** onto the side of the well.
	Proper **Elution Buffer** was not used	The provided **Elution Buffer** has been optimized for high elution recoveries. If water is used, ensure that the pH is between 7 and 8.
DNA does not perform well in downstream applications	DNA was not washed with the provided **Wash Solution**	Traces of salt from the binding step may remain in the sample if the column is not washed with **Wash Solution**. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
	A different **Elution Buffer** was used	If a different **Elution Buffer** other than the one provided in the kit was used, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.

Related Products	Product #
Plasmid MiniPrep DNA Kit	13300, 46400
Plasmid DNA Maxiprep Kit	46500, 46600
Low Copy Plasmid DNA MiniPrep Kit	17800
BAC DNA Miniprep Kit	18050

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
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Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

