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 MaxiPrep Kit

Product Insert

Product # 46500, 46600

Norgen's Plasmid DNA MaxiPrep Kit is designed for the rapid preparation of plasmid DNA from up to 100 mL of *Escherichia coli* culture. The kit allows for the isolation of plasmid DNA with final endotoxin levels of 0.1 EU/μg of DNA or less. The kit is able to purify plasmids up to 13,000 bp in size, and typical yields from a 100 mL culture for a high copy number plasmid are between 0.4 and 1.0 mg. 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 spin column chromatography. The plasmid DNA is preferentially purified from other cellular components such as genomic DNA and RNA. The process involves first pelleting overnight bacteria culture harbouring plasmid DNA (please see flow chart on page 3). The bacterial pellet is then resuspended using the provided Resuspension Solution AZ, and the bacteria are lysed using the Lysis Buffer N. Buffer TN is then added, causing the genomic DNA and proteins present in the solution to precipitate. The lysate is then clarified through centrifugation, in order to remove the precipitated proteins and genomic DNA from the lysate containing the plasmid DNA. The clarified lysate is then loaded onto a spin-column. Norgen's column binds DNA in a manner that depends on ionic concentrations, thus only the plasmid DNA will bind to the column while most of the digested RNA and proteins will be removed in the flowthough or retained on top of the column bed. The bound DNA is then washed with the provided Wash Solution J in order to remove any remaining impurities. Lastly, the endotoxin-free purified plasmid DNA is eluted with the Elution Buffer J. The purified DNA is of the highest quality and can be used in a number of downstream applications including sequencing, cloning, and transfections.

Specifications

Kit Specifications	
Column Binding Capacity	1.5 mg
Average Yield from 100 mL Culture	0.4 – 1.0 mg
Final Endotoxin Levels	≤ 0.1 EU/μg DNA
Time to Complete 4 Purifications	1.5 hours
Size of Plasmids Purified	Up to 13,000 bp

Advantages

- Isolate up to 1 mg of high copy number plasmid DNA from 100 mL of culture
- Endotoxin-free plasmid DNA - levels of ≤ 0.1EU/μg of plasmid DNA
- Fast and easy processing using a rapid spin-column format
- Isolate high quality plasmid DNA

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. The **RNase** vial should be stored at -20°C upon arrival. Once RNase has been added to the **Resuspension Solution AZ** the solution should be stored at 4ºC. This kit is stable for 1 year after its date of shipment.

Kit Components

Component	Product # 46500 (4 samples)	Product # 46600 (20 samples)
Resuspension Solution AZ	20 mL	100 mL
Lysis Buffer N	40 mL	2 x 80 mL
Buffer TN	55 mL	2 x 130 mL
Wash Solution J	25 mL	3 x 25 mL
Elution Buffer J	24 mL	120 mL
RNase	1 vial	1 vial
DNA Maxi Spin Columns with Collection Tubes (Clear ring in column)	4	20
Maxi Spin Filter Columns with Collection Tubes (Grey ring in column)	4	20
Elution Tubes (50 mL)	4	20
Product Insert	1	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*.

Buffer TN 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

- Centrifuge with a swinging bucket rotor capable of 3000 x g
- 250 mL centrifuge tubes
- Centrifuge capable of 14 000 x g
- 96 - 100% ethanol

Procedure

Various speeds are required for the different centrifugation steps, so please check your centrifuge specifications to ensure that it is capable of the proper speeds. 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. All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.

Flow Chart

Procedure for Purifying Plasmid DNA Norgen's Plasmid DNA MaxiPrep Kit

Pellet from overnight bacterial culture harbouring high copy plasmid

Add Resuspension Solution A,
Lysis Buffer N,
Buffer TN

SPIN and FILTER

Transfer lysate

Bind to Column

SPIN

Wash with Wash Solution J

SPIN

Dry Spin

Elute DNA with
Elution Buffer J

SPIN

Pure Plasmid DNA

Notes prior to use:

- Ensure that all solutions, except the **Resuspension Solution AZ,** 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.
- Prepare a working concentration of **Wash Solution J** by adding 100 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottles containing concentrated **Wash Solution J**. This will give a final volume of 125 mL. The labels on the bottles have a box that can be checked to indicate that ethanol has been added.
- Take the entire amount of **RNase** and add it to the **Resuspension Solution AZ**. 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.
- Bacterial cultures grown overnight at 37°C in LB medium are optimal for this procedure
- This kit contains both Maxi Filter Spin Columns and DNA Maxi Spin Columns provided in Elution Tubes. When columns are removed from the labelled bags they are supplied in they can easily be identified as follows:
    - Maxi Filter Spin Columns – Grey ring inside of column
    - DNA Maxi Spin Columns – Clear ring inside of column

1. Lysate Preparation

a. Transfer 100 mL of an overnight bacterial culture to a centrifuge tube and centrifuge at 6,000 x g (~6,000 RPM) for 15 minutes to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. Add 4 mL of **Resuspension Solution AZ** (containing **RNase**; see Notes Prior to Use) to the cell pellet. Resuspend the cells by pipetting in and out, or by gentle vortexing. Make sure that the pellet is completely resuspended. Incubate at room temperature for 15 minutes.

c. Add 7 mL of **Lysis Buffer N** 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.

Note: 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 12 mL of **Buffer TN** 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 10 minutes at 14,000 x g (~14,000 RPM) to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.

f. Transfer the lysate to a provided **Maxi Spin Filter Column with Collection Tube** (grey ring in column) and centrifuge in a swinging bucket rotor centrifuge for 5 minutes at 3,000 x g (~3,000 RPM). The cap must be placed loosely onto the filter column assembly since tightly capped columns will impede liquid flow.

The filter column will remove any floating particles in the lysate not clarified during the previous centrifugation step. Ensure that the bulk pellicle is not transferred to the filter column assembly.

2. Binding to Column

a. Apply the clarified lysate to the reservoir of the **DNA Maxi Spin Column with Collection Tube** (clear ring in column). Again, place the cap loosely onto the column assembly so as not to impede liquid flow.

b. Using a swinging bucket rotor centrifuge, spin the assembly at 1,000 x g (~1,000 RPM) for 5 minutes. Discard the flowthrough and reassemble the spin column with its collection tube.

Note: Ensure the entire lysate has passed through into the collection tube by inspecting the column. If the entire volume has not passed, spin for an additional 5 minute at 3,000 x g (~3,000 RPM).

3. Washing Bound DNA

a. Apply 15 mL of **Wash Solution J** to the column assembly and centrifuge the unit for 10 minutes at 3,000 x g (~3,000 RPM). Discard the flowthrough and reassemble the unit.

b. Centrifuge the column assembly for 5 minutes at 3,000 x g (~3,000 RPM). Discard the collection tube.

4. Elution of Clean DNA

a. Assemble the column with a fresh 50 mL **Elution Tube** provided with the kit.

b. Add 2 mL of **Elution Buffer J** to the center of the column bed and centrifuge the column assembly at 3,000 x g (~3,000 RPM) for 5 minutes.

Related Products	**Product #**
Plasmid MiniPrep Kit	13300, 46400
PCRSizer 100bp DNA Ladder	11300
HighRanger 1kb DNA Ladder	11900
Plasmid MiniPrep Kit (Magnetic Bead System)	60300, 63000

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.

Troubleshooting Guide

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.
	Lysate was prepared incorrectly	The **Lysis Buffer N** may have formed precipitates. Warm and mix gently before use.
	Cell resuspension was incomplete	Pelleted cells should be completely resuspended in the **Resuspension Solution AZ**. Do not add **Lysis Buffer N** until a homogeneous suspension is obtained.
DNA does not perform well in downstream applications	DNA was not washed with the provided **Wash Solution J**	Traces of salt from the binding step may remain in the sample if the column is not washed with the **Wash Solution J**. Salt may interfere with downstream applications, and thus must be washed from the column.
	Dry spin not performed	The dry spin must be performed after the wash step in order to remove all traces of ethanol.
	The appropriate amount of ethanol was not added to the **Wash Solution J**	The **Wash Solution J** has been specifically designed to contain the appropriate amount of components. Ensure that the **Wash Solution J** was prepared using the correct amount of ethanol.
	A different Elution Buffer was used	The provided **Elution Buffer J** has been optimized for endotoxin-free recoveries. The endotoxin-free properties of the eluted DNA will be compromised if another elution buffer is used. If a different **Elution Buffer J** other than the one provided is used, the buffer should also 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.

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