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Plasmid DNA MaxiPrep Kit (Endotoxin Free) Product Insert

Product # 15300

Norgen's Plasmid DNA MaxiPrep Kit (Endotoxin Free) is designed for the rapid preparation of endotoxin-free plasmid DNA from up to 100 mL of *Escherichia coli* culture. Endotoxins are a class of lipopolysaccharides which are an integral part of the outer cell membrane of Gram-negative bacteria. Endotoxins are released during the lysis step of plasmid purification, and have been found to significantly reduce transfection efficiencies in endotoxin sensitive cell lines. Therefore, the removal of endotoxins during large scale plasmid isolation is essential when the plasmid DNA is going to be used for transfections. Norgen's Plasmid DNA MaxiPrep Kit (Endotoxin Free) 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 using Norgen's proprietary resin as the separation matrix. 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, and the bacteria are lysed using the Lysis Solution. Neutralization Solution 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 transferred to a new tube, and both Binding Solution and Endotoxin Removal Solution are added. Isopropanol is then added to the mixture, and the solution is loaded onto a spin-column. Norgen's resin 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 resin. The bound DNA is then washed once with the provided wash buffer and once with the elution buffer in order to remove any remaining impurities. Lastly, the endotoxin-free purified plasmid DNA is eluted with the elution buffer. 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

Kit Components

Component	Product # 15300 (4 samples)
Resuspension Buffer	30 mL
Lysis Solution	30 mL
Neutralization Solution	30 mL
Binding Solution	10 mL
Wash Solution	40 mL
Elution Buffer	20 mL
Endotoxin Removal Solution	1 mL
RNAse	26500 units
Maxi Spin Columns (assembled with collection tubes)	4
Filter Columns (assembled with collection tubes)	4
Elution Tubes (50 mL)	4
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. The **RNAse** should be stored at -20°C upon arrival. Once RNAse has been added to the **Resuspension Buffer**, however, the solution should be stored at 4^{o}C. All the reagents should remain stable for at least 1 year in their unopened containers.

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*.

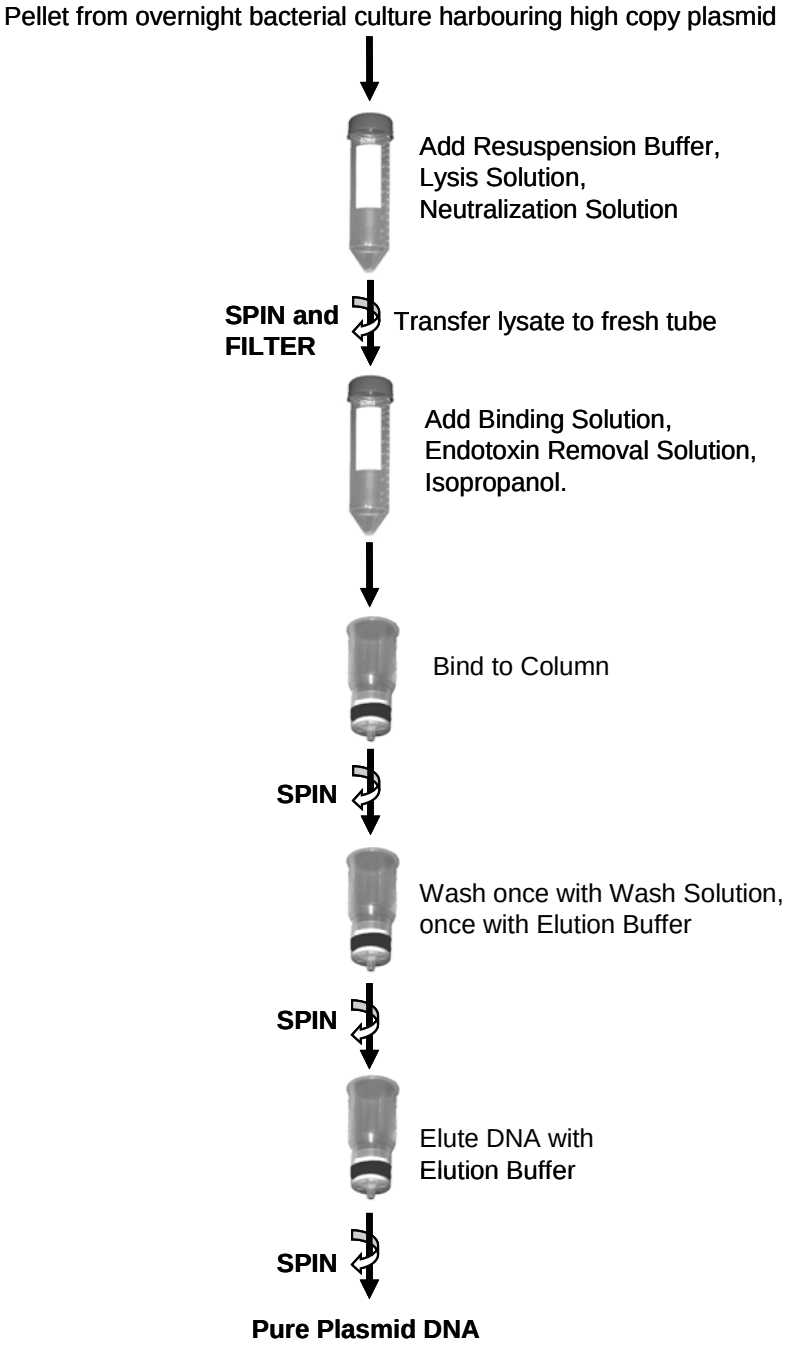
The **Binding Solution** contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Customer-Supplied Reagents and Equipment

- 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
- Isopropanol

Flow Chart

Procedure for Purifying Plasmid DNA Norgen's Plasmid DNA MaxiPrep Kit (Endotoxin Free)



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.

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 40 mL of 96 - 100% ethanol to the **Wash Solution**. Mix well by inversion.
- Take the entire amount of **RNAse** and add it 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.
- Bacterial cultures grown overnight at 37°C in LB medium are optimal for this procedure.

1. Lysate Preparation

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

b. Add 6 mL 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 15 minutes.

c. Add 6 mL 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.

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 6 mL of **Neutralization 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 10 minutes at 14,000 x g to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.

f. Transfer the lysate to the provided filter column assembly and centrifuge in a swinging bucket rotor centrifuge for 5 minutes at 3,000 x g. 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.** Add 2 mL of **Binding Solution** to the lysate and mix well.
- **b.** Add 200 μL of **Endotoxin Removal Solution** to the lysate and mix well by inversion and vortexing. Let stand for 5 minutes. (Caution: Solution is very viscous; be careful pipetting)
- **c.** Add 2mL of **Isopropanol** and mix thoroughly by vortexing.
- **d.** Apply all of the lysate to the reservoir of the spin column assembly. Again, place the cap loosely onto the column assembly so as not to impede liquid flow.
- **e.** Using a swinging bucket rotor centrifuge, spin the assembly at 3,000 x g for 5 minutes. Discard the flowthrough and reassemble the spin column with its collection tube.

3. Washing Bound DNA

- **a.** Apply 15 mL of **Wash Solution** to the column assembly and centrifuge the unit for 10 minutes at 3,000 x g. It is not necessary to discard the flowthrough and reassemble the unit since the collection reservoir can accommodate up to 22 mL of liquid.
- **b.** Add 0.5 mL of **Elution Buffer** to the column unit ensuring the top frit is evenly covered. Centrifuge the column assembly for 5 minutes at 3,000 x g. Discard the collection tube.

  Passage of a small amount of elution buffer through the column before the purified DNA is eluted allows for the recovery of a higher concentration of purified plasmid DNA.

4. Elution of Clean DNA

- **a.** Assemble the column (with DNA bound to the resin) with a fresh 50 mL **Elution Tube** provided with the kit.
- **b.** Add 2 mL of **Elution Buffer** to the center of the resin bed and centrifuge the column assembly at 3,000 x g for 5 minutes.

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 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.
DNA does not perform well in downstream applications	DNA was not washed twice with the provided **Wash Solution** and **Elution Buffer**	Traces of salt from the binding step may remain in the sample if the column is not washed twice; once with the **Wash Solution** and once with the **Elution Buffer**. Salt may interfere with downstream applications, and thus must be washed from the column.
	The appropriate amount of ethanol was not added to the **Wash Solution**	The **Wash Solution** has been specifically designed to contain the appropriate amount of components. Ensure that the **Wash Solution** was prepared using the correct amount of ethanol.
	A different **Elution Buffer** was used	The provided **Elution Buffer** 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** 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.

Related Products	Product #
Plasmid MiniPrep Kit	13300
Low Copy Plasmid MiniPrep Kit	17800
BAC DNA MiniPrep Kit	18000
PCRSizer 100bp DNA Ladder	11300
HighRanger 1kb DNA Ladder	11900

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.

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