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Endotoxin Removal Kit (Midi) Product Insert

Product # 52200

Norgen's **Endotoxin Removal Kit (Midi)** is designed for the rapid removal of endotoxins from up to 200 μg of previously purified DNA. Endotoxins, also known as lipopolysaccharides, are cell-membrane components of Gram-negative bacteria such as *E. coli*. Endotoxins are released during the lysis step of plasmid purification and significantly reduce transfection efficiencies in endotoxin sensitive cell lines. Therefore, the removal of endotoxins from plasmid preparations is often necessary prior to the use of the DNA in downstream applications. With Norgen's Endotoxin Removal Kit (Midi), endotoxin levels are efficiently reduced to 0.1 EU/μg DNA or less. Each kit contains sufficient materials for 10 purifications, and preparation time for a single sample is approximately 30 minutes.

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 the contaminating endotoxins with this kit. The first step in the process involves the addition of Buffer SK to the DNA sample (please see flow chart on page 3). The sample is then placed into the top reservoir of the column, and a small amount of Endotoxin Removal Solution is added. After a brief incubation, isopropanol is also added to the column and the solution is mixed and then spun in a centrifuge. Norgen's resin binds DNA in a manner that depends on ionic concentrations, thus only the plasmid DNA will bind to the column while the contaminating endotoxins will be removed in the flowthough. The bound DNA is then washed with the provided wash buffer to remove any remaining impurities. Lastly, the endotoxin-free plasmid DNA is eluted with Elution Buffer F. 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	
Maximum DNA Input	200 μg
Maximum DNA Volume Input	0.75 mL
Final Endotoxin Levels	≤ 0.1 EU/μg DNA
Time to Complete 4 Purifications	30 minutes
Average Recovery	> 90% (~ 80% in First Elution)

Advantages

- Endotoxin-free DNA - reduce endotoxin levels to 0.1EU/μg of plasmid DNA or less
- Fast and easy processing using a rapid spin-column format
- High recovery of input DNA – recovery is greater than 90%

Kit Components

Component	Product # 52200 (10 samples)
Buffer SK	60 mL
Wash Solution H	18 mL
Elution Buffer I	12 mL
Endotoxin Removal Solution	1.5 mL
Precipitation Solution	1.5 mL
Spin Columns (assembled with collection tubes)	10
Elution Tubes	10
Product Insert	1

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

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 **Buffer SK** contains guanidine thiocyanate, and should be handled with care. Guanidine thiocyanate 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
- 15 mL conical tubes
- 96 – 100% ethanol
- 70% ethanol
- Isopropanol

Procedure
All centrifugation steps are carried out in a benchtop centrifuge. Various speeds are required for different 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:

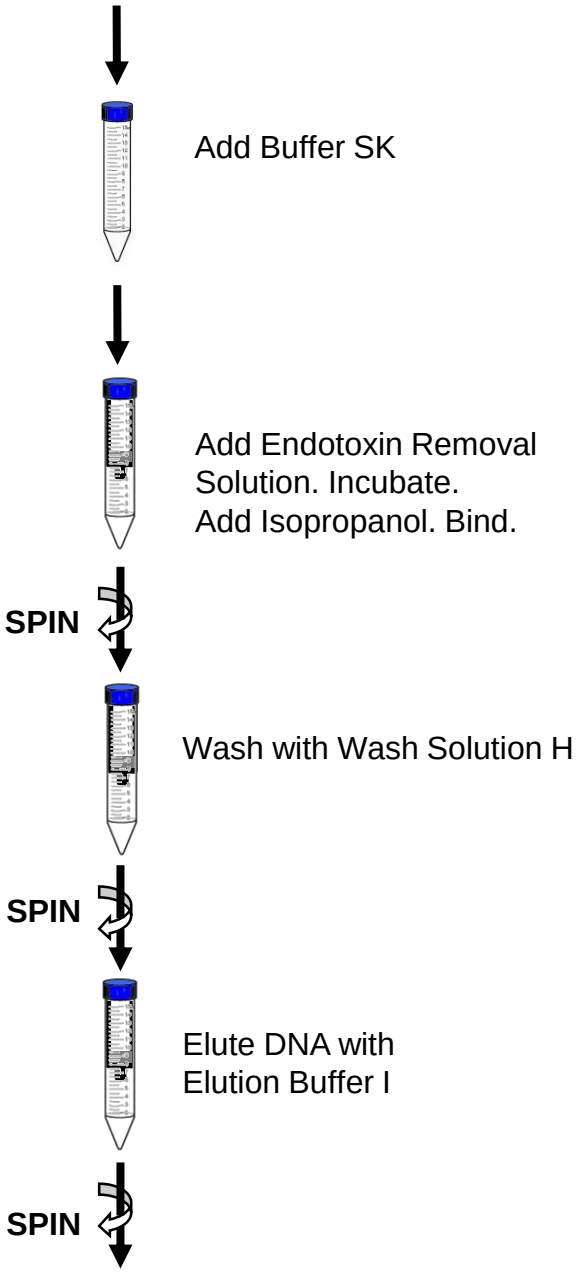
$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\ (r)}}$$

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 Removing Endotoxins using Norgen's Endotoxin Removal Kit (Midi)

Obtain previously purified plasmid DNA sample



Endotoxin - Free Plasmid DNA

Notes prior to use:

- Ensure that all solutions 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 H** by adding 42 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution H**. 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.
- **Ensure that the maximum DNA input does not exceed 200 μg or 0.75 mL.** If the amount of DNA or the volume exceeds this, the sample will need to be processed using more than 1 column.

1. Sample Preparation

a. Transfer up to 0.75 mL of DNA into a 15 mL conical tube. Add 5 volumes of **Buffer SK** to the DNA and mix well by inversion or vortexing.

Note: For example, add 2.5 mL of **Buffer SK** to 0.5 mL of DNA. The total volume should not exceed 4.5 mL.

b. Obtain a column inserted into a collection tube. Add the DNA solution to the top of the column.

c. Add a 1% volume of **Endotoxin Removal Solution** to the liquid on top of the column. Pipette up and down to mix. Let stand for 5 minutes at room temperature.

Note: For example, if the volume of DNA solution from **1a** is 3 mL, add 30 μL of **Endotoxin Removal Solution**.

d. After 5 minutes, add a 10% volume of isopropanol to the liquid on the column. Pipette up and down to mix.

Note: For example, add 300 μL of isopropanol to the 3.03 mL solution from above.

2. Binding to Column

a. Spin the column at 3,200 x g (~4,000 RPM) for 5 minutes in a benchtop centrifuge.

b. Discard the flowthrough and reassemble the spin column with its collection tube.

3. Washing Bound DNA

a. Apply 5 mL of **Wash Solution H** to the column assembly and centrifuge the unit for 10 minutes at 3,200 x g (~4,000 RPM).

b. Discard the flowthrough and reassemble the unit.

c. Spin the column for an additional 10 minutes at 3,200 x g (~4,000 RPM) in order to completely remove all traces of ethanol from the resin.

4. Elution of Clean DNA

a. Assemble the column (with DNA bound to the resin) with a fresh 15 mL **Elution Tube** provided with the kit.

b. Add 0.5 mL of **Elution Buffer I** to the center of the resin bed and centrifuge the column assembly for 5 minutes at 3,200 x g (~4,000 RPM).

Note: About 70 to 80 % of the input amount will be recovered in the first elution. However, a second elution may be performed if desired. This will recover an additional 20 to 30% of the input amount. Step **4b** should be repeated, and the elution should be collected into a fresh elution tube in order to prevent dilution of the first elution.

Optional Concentration of Eluted DNA
If the concentration of the eluted DNA is found to be too dilute, the DNA can be concentrated using the following protocol:

a. Transfer eluted DNA to a centrifuge tube.
b. Add 50 µL of **Precipitation Solution** to the 0.5 mL eluted DNA sample.
c. Add 1.5 mL of **COLD** 96-100% ethanol to the DNA. Mix well.
d. Place DNA at -20ºC or -70ºC for a minimum of 30 minutes (overnight if preferred).
e. Centrifuge at 14,000 x g (~14,000 RPM) for 20 minutes and discard supernatant.
f. Wash the DNA pellet with 1 mL of 70% ethanol. Centrifuge at 14,000 x g (~14,000 RPM) for 10 minutes and discard supernatant.
g. Allow pellet to air dry.
h. Resuspend pellet in the desired volume of the provided **Elution Buffer I**.

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	DNA did not bind properly to the column	Ensure that the **Buffer SK** does not contain any precipitates. Warm and mix gently if necessary.
	The appropriate amount of ethanol was not added to **Wash Solution H**	**Wash Solution H** has been specifically designed to contain the appropriate amount of components. Ensure that **Wash Solution H** is prepared using the correct amount of ethanol.
	Incomplete removal of **Wash Solution H**	Ensure that the column is spun for 3 minutes after the first wash step, in order to completely dry the column. Traces of ethanol may remain in the eluted sample otherwise, and interfere with subsequent enzymatic reactions.
	The appropriate amount of **Buffer SK** was not added	Ensure that 2.5 mL of **Buffer SK** is added for every 0.5 mL of DNA processed. The DNA volume must not exceed 0.75 mL.

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications	DNA was not washed with the provided **Wash Solution H**	Ensure that the column is washed with the appropriate amounts of **Wash Solution H**. This solution has been specifically designed to remove all traces of ethanol and endotoxin.
	Proper Elution Buffer was not used	The provided **Elution Buffer I** has been optimized for endotoxin-free recoveries. If endotoxin-free water is used for the elution, ensure that the pH is between 7 and 8.
	A different Elution Buffer was used	The provided **Elution Buffer I** 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.
Endotoxin levels in the eluted DNA are slightly higher than 0.1 EU/μg DNA	A different Elution Buffer was used	The provided **Elution Buffer I** 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 endotoxin levels.
	The endotoxin levels of the input were extremely high	If the initial input DNA had extremely high endotoxin levels, the levels may not be completely reduced to 0.1 EU/μg of DNA or less. In this case, the eluted DNA could be applied to a second column and the procedure repeated in order to further reduce the endotoxin levels.

Related Products	Product #
Plasmid MaxiPrep Kit (Endotoxin Free)	15300
Endotoxin Removal Kit (Mini)	22700
Endotoxin Removal Kit (Maxi)	21900

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