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Endotoxin Removal Kit — Product Insert

Product # 21900

Norgen's **Endotoxin Removal Kit** is designed for the rapid removal of endotoxins from up to 1 mg 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, endotoxin levels are efficiently reduced to 0.1 EU/μg DNA or less. Each kit contains sufficient materials for 4 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 Binding Solution 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 once with the provided wash buffer to remove any remaining impurities. Lastly, the endotoxin-free 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	
Maximum DNA Input	1 mg
Maximum DNA Volume Input	3 mL
Final Endotoxin Levels	≤ 0.1 EU/μg DNA
Time to Complete 4 Purifications	30 minutes
Average Recovery	> 90%

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 # 21900 (4 samples)
Binding Solution	60 mL
Wash Solution	18 mL
Elution Buffer	12 mL
Endotoxin Removal Solution	1 mL
Precipitation Solution	1.5 mL
Spin Columns (assembled with collection tubes)	4
Elution Tubes	4
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. All the reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers

User must determine the suitability of the product for their particular use. The kit is intended for research purposes only and not for human or drug use. The kit is not designed for diagnostic purposes. MSDS sheets are available upon request.

The **Binding Solution** 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.

Ensure that lab coats and gloves are worn when working with this kit.

Customer-Supplied Reagents and Equipment

- Centrifuge with a swinging bucket rotor capable of 3000 x g
- 50 mL conical tubes
- 96 – 100% ethanol
- Isopropanol

Flow Chart

Procedure for Removing Endotoxins using Norgen's Endotoxin Removal Kit

Obtain previously purified plasmid DNA sample

Add Binding Solution

Add Endotoxin Removal Solution. Incubate. Add Isopropanol. Bind.

SPIN

Wash DNA with Wash Solution

SPIN

Elute DNA with Elution Buffer

SPIN

Endotoxin-Free Plasmid DNA

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.

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** by adding 42 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. Do not use denatured alcohol, as this can lead to precipitation of salts.
- **Ensure that the maximum DNA input does not exceed 1 mg or 3 mL.** If the amount of DNA or the volume exceeds this, the sample will need to be processed using more than 1 column.
- **The minimum binding volume must be at least 5 mL.** The input DNA can be diluted in Milli-Q water if necessary in order to increase the final binding volume to 5 mL.

1. Sample Preparation

a. Transfer up to 3 mL of DNA into a 50 mL conical tube. Add 5 volumes of **Binding Solution** to the DNA and mix well by inversion or vortexing.

Note: For example, add 5 mL of **Binding Solution** to 1 mL of DNA. The total volume should not exceed 18 mL.

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

c. Add 1% volume of **Endotoxin Removal Solution** to the liquid on top of the column. Close the lid and vortex gently to mix. Let stand for 5 minutes at room temperature.

Note: For example, if the volume of DNA solution from **1a** is 6 mL, add 60 μL of **Endotoxin Removal Solution**.

d. After 5 minutes, add a 10% volume of isopropanol to the liquid on the column. Close lid and vortex gently to mix.

Note: For example, add 600 μL of isopropanol to the 6.06 mL solution from above.

2. Binding to Column

a. Spin the column at 3,000 x g 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 15 mL of **Wash Solution** to the column assembly and centrifuge the unit for 10 minutes at 3,000 x g.
b. Discard the flowthrough and reassemble the unit.
c. Spin the column for an additional 3 minutes at 3,000 x g, in order to completely dry the resin. Discard the collection tube.

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 1 mL of **Elution Buffer** to the center of the resin bed and centrifuge the column assembly for 5 minutes at 3,000 x g.

Note: Greater than 90% of the input amount will be recovered in the first elution. However, a second elution may be performed if desired. Steps 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
The concentration of the eluted DNA may be found to be too dilute. If this is the case, the DNA can be concentrated using the following protocol:

a. Transfer eluted DNA to a microcentrifuge tube.
b. Add 100 μL of **Precipitation Solution** to the eluted DNA sample.
c. Add 3 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 for 20 minutes and discard supernatant.
f. Allow pellet to air dry.
g. Resuspend pellet in the desired volume of the provided **Elution Buffer**.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	DNA did not bind properly to the column	Ensure that the **Binding Solution** does not contain any precipitates. Warm and mix gently if necessary.
	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.
	The appropriate amount of **Binding Solution** was not added	Ensure that 5 mL of **Binding Solution** is added for every 1 mL of DNA processed. The DNA volume must not exceed 3 mL.
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.
	Proper **Elution buffer** was not used	The provided **Elution Buffer** 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** 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** 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)	21800

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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