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

Endotoxin Removal Kit (Mini) Product Insert

Product # 22700

Norgen's **Endotoxin Removal Kit (Mini)** is designed for the rapid removal of endotoxins from up to 25 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 (Mini) endotoxin levels are efficiently reduced to 0.1 EU/μg DNA or less. Each kit contains sufficient materials for 25 purifications, and preparation time for a single sample is approximately 20 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 microcentrifuge. 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 Solution H to remove any remaining impurities. Lastly, the endotoxin-free plasmid DNA is eluted with the Elution Buffer I. 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	25 μg
Maximum DNA Volume Input	100 μL
Final Endotoxin Levels	≤ 0.1 EU/μg DNA
Time to Complete 10 Purifications	20 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%

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.

Kit Components

Component	Product # 22700 (25 samples)
Buffer SK	15 mL
Wash Solution H	18 mL
Elution Buffer I	6 mL
Endotoxin Removal Solution	1.5 mL
Spin Columns	25
Collection Tubes	25
Elution Tubes (1.7 mL)	25
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*.

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

- Benchtop microcentrifuge
- Microcentrifuge tubes
- 95-100% ethanol
- Isopropanol

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. 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 (Mini)

Obtain previously purified plasmid DNA sample

Add Buffer SK

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

SPIN

Wash DNA with Wash Solution H

SPIN

Elute DNA with Elution Buffer I

SPIN

Endotoxin-Free Plasmid DNA

Notes prior to use:

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- 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 25 μg or 100 μL.** 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 100μL of DNA into a microcentrifuge tube. Add 5 volumes of **Buffer SK** to the DNA and mix well by inversion or vortexing.

Note: For example, add 500 μL of **Buffer SK** to 100 μL of DNA. The total volume should not exceed 600μL.

b. Assemble a spin column with a provided 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 column assembly **gently** to mix. Let stand for 5 minutes at room temperature.

Note: For example, if the volume of DNA solution from **1a** is 600 μL, add 6μL of **Endotoxin Removal Solution**.

If dripping occurs into the collection tube during the 5 minute incubation period, just proceed with protocol as written.

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

Note: For example, add 60 μL of isopropanol to the 606 μL solution from above.

2. Binding to Column

a. Spin the column at 14,000 x g (~14,000 RPM) for 1 minute in a microcentrifuge.

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

3. Washing Bound DNA

a. Apply 500 μL of **Wash Solution H** to the column assembly and centrifuge the unit for 2 minutes at 14,000 x g (~14,000 RPM).

b. Discard the flowthrough and reassemble the unit.

c. Spin the column for an additional 1 minute at 14,000 x g (~14,000 RPM), 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 1.7 mL **Elution Tube** provided with the kit.
   - **b.** Add 100 µL of **Elution Buffer I** to the center of the resin bed and centrifuge the column assembly for 2 minutes at 200 x g (~2,000 RPM).
   - **c.** Without removing the assembly, centrifuge for 1 minute at 14,000 x g (~14,000 RPM) to elute the DNA.

   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 and 4c should be repeated, and the elution should be collected into a fresh elution tube, in order to prevent dilution of the first elution. The first elution volume can be reduced to as little as 50 µL if desired.

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 the **Wash Solution H**	The **Wash Solution H** has been specifically designed to contain the appropriate amount of components. Ensure that the **Wash Solution H** was prepared using the correct amount of ethanol.
	The appropriate amount of **Buffer SK** was not added	Ensure that 5µL of **Buffer SK** is added for every 1 µL of DNA processed. The DNA volume must not exceed 100 µL.
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.

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications	DNA was not washed with the provided **Wash Solution H**	Traces of salt from the binding step may remain in the sample if the column is not washed with **Wash Solution H**. Salt may interfere with downstream applications, and thus must be washed from the column.
	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.

Related Products	Product #
Plasmid MaxiPrep DNA Kit	46500
Endotoxin Removal Maxi Kit	21900
Endotoxin Removal Midi Kit	52200

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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