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ProteoSpin™ Endotoxin Removal Maxi Kit — Product Insert

Product # 22200

The ProteoSpin™ Endotoxin Removal Maxi Kit is designed for the rapid removal of endotoxins from up to 4 mg of previously purified proteins or peptides. Endotoxins, also known as lipopolysaccharides, are cell-membrane components of Gram-negative bacteria such as *E. coli*. Endotoxins liberated by Gram-negative bacteria are frequent contaminations of protein solutions derived from bioprocesses. Due to the high toxicity of endotoxins *in vivo* and *in vitro*, their removal from protein preparations is often necessary prior to the use of the protein in downstream applications. The ProteoSpin™ Endotoxin Removal Maxi Kit efficiently reduces endotoxin levels to ≤ 0.1 EU/μg of protein using spin column chromatography based on Norgen's proprietary resin. The purified protein samples can be used in a number of downstream applications including in vitro and in vivo introduction into cells and organisms, sequencing, and cloning.

The ProteoSpin™ Endotoxin Removal Maxi Kit contains sufficient materials for 4 purifications. Each column is able to remove endotoxins from up to 4 mg of previously purified proteins, with protein recoveries of > 95% being achieved. Preparation time for a single sample is approximately 30 minutes.

Specifications

Kit Specifications	
Maximum Protein Input	4 mg
Maximum Initial Protein Volume Input	18 mL
Final Endotoxin Levels	≤ 0.1 EU/μg protein
Time to Complete 10 Purifications	30 minutes
Average Recovery	>95 %

Kit Components

Component	Product # 22200 (4 samples)
Binding Buffer J	8 mL
Wash Solution M	130 mL
Elution Buffer G	20 mL
Endotoxin Removal Solution	1.5 mL
Protein Neutralizer EF	2 mL
Maxi Spin 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. 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*.

Customer-Supplied Reagents and Equipment

- Centrifuge with a swinging bucket rotor capable of 3,000 x g
- 50 mL conical tubes
- Isopropanol
- Sterile, deionized water or Milli-Q® water

Procedure

All centrifugation steps are carried out at 1,000 x g in a benchtop centrifuge. Please check your centrifuge specifications to ensure proper speed. Performance of the kit is not affected by temperature, and thus the procedure may be performed at room temperature, 4°C, or on ice.

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.
- Ensure that the initial protein input does not exceed 4 mg or 18 mL**.** If the mass or volume of protein exceeds this, the sample will need to be processed using more than 1 column.
- High molarities of salts will interfere with the pH adjustment in the Sample Preparation step. Dilute the molarity of the input protein solution to < 50 mM using sterile, deionized water or Milli-Q® water. If the volume increases over the maximum 18 mL input, the sample can be split and processed using 2 columns.

1. Column Activation

a. Obtain a spin column with its 50 mL conical collection tube. Remove lid.
b. Apply 5 mL of **Wash Solution M** to the column and close the cap.
c. Centrifuge for 2 minutes at 1,000 x g.
d. Repeat steps **1b** and **1c,** and discard the flowthrough to complete the column activation step.

2. Sample Preparation

a. Obtain the protein sample and measure the volume. If the volume is not at least 5 mL, bring the volume up to 5 mL using sterile, deionized water or Milli-Q® water.
b. Add 40 µL of **Binding Buffer J** for every millilitre of protein solution and mix well.
c. Verify that the pH is between 3.5 and 4, and add more Binding Buffer J if necessary.
d. Transfer the protein sample into the top reservoir of the activated column (from Step 1 above).
e. Add a 1% volume of **Endotoxin Removal Solution** to the liquid on top of the column. (i.e.: Add 50 µL for every 5 mL volume of input placed onto the column).

Note: The Endotoxin Removal Solution is extremely viscous, and thus care should be taken when measuring out the correct volume.

f. Close the lid of the column and vortex the column assembly **GENTLY** to mix. Let stand for 5 minutes at room temperature.

Note: Some dripping of the protein sample into the collection tube may occur during the incubation period. Even if this does occur, proceed with the protocol as written.

g. After the 5 minute incubation, add a 10% volume of **isopropanol** (supplied by the user) to the liquid on the top of the column. Close the lid and vortex column assembly **GENTLY** to mix. (i.e.: Add 500 µL of isopropanol for every 5 mL of sample on top of the column).

3. Binding to Column

a. Place the column into a benchtop centrifuge and spin at 1,000 x g for 5 minutes.
b. Discard the flowthrough and reassemble the spin column with its collection tube.

Note: If all of the liquid does not pass through into the collection tube, repeat steps **3a** and **3b.**

4. Washing Bound Protein

a. Apply 10 mL of **Wash Solution M** to the column assembly and centrifuge the unit for 3 minutes at 1,000 x g.
b. Discard the flowthrough and reassemble the spin column with the collection tube.
c. Repeat steps **4a** and **4b**.
d. Spin the column for an additional 2 minutes at 1,000 x g in order to completely dry the resin. Discard the collection tube.

5. Elution of Clean Protein

a. Add 140 µL of **Protein Neutralizer EF** to a provided Elution tube**.**
b. Assemble the column (with protein bound to the resin) with the Elution tube from above.
c. Add 2 mL of **Elution Buffer G** to the center of the resin bed and centrifuge the column assembly for 5 minutes at 3,000 x g.

Note: The majority of the input proteins will be recovered in the first elution. However, a second elution may be performed if desired. Steps 5a to 5c should be repeated, and the elution should be collected into a fresh elution tube in order to prevent dilution of the first elution. The total elution volume can also be reduced to 1 mL if required, but the amount of Protein Neutralizer EF must be reduced to 70 µL as well.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Protein solution does not flow through the column	Centrifugation speed was too low	Check the centrifuge and ensure that it is capable of generating 1,000 x g. Sufficient centrifugal force is required to push the liquid through the column.
	Inadequate spin times	Spin for an additional 5 minutes to ensure that the liquid is able to flow through the column.
	Cellular debris is present in the protein solution	Prior to the sample preparation step, filter the sample with a 0.45 μM filter or spin down insoluble materials. Solid, insoluble materials can cause clogging problems.
Poor Protein Recovery	Input protein solution is very high in molarity.	The **Binding Buffer J** may not lower the pH of the protein solution sufficiently if the input protein solution has a very high molarity. Dilute the molarity of the input to < 50mM with water. If the volume of the input amount is increased over the maximum 18 mL input, the sample can be split and processed using 2 columns
	The appropriate amount of **Binding Buffer J** was not added	Ensure that 40 μL of **Binding Buffer J** is added for every 1 mL of protein processed. The initial protein volume must not exceed 18 mL.
Protein does not perform well in downstream applications	A different Elution Buffer was used	The provided **Elution Buffer G** has been optimized for endotoxin-free recoveries. The endotoxin-free properties of the eluted protein will be compromised if another Elution Buffer G is used. If a different **Elution Buffer G** 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, detergents and other denaturants. Check the compatibility of your Elution Buffer G with the intended use.
Endotoxin levels are high in the elution	A different Elution Buffer was used	The provided **Elution Buffer G** has been optimized for endotoxin-free recoveries. The endotoxin-free properties of the eluted protein will be compromised if another Elution Buffer G is used. If a different **Elution Buffer G** other than the one provided is used, the buffer should also be checked for Endotoxin levels.
	Extremely high endotoxin levels in input protein	Check the endotoxin levels of your protein input. The endotoxin levels of the eluted protein may be reduced significantly compared to the input.

Problem	Possible Cause	Solution and Explanation
Eluted protein is degraded	Eluted protein was not neutralized	Add 140 µL of Protein Neutralizer EF for every 2 mL of eluted protein in order to adjust the pH to neutral. Some proteins are sensitive to high pH, such as the Elution Buffer G.
	Bacterial contamination of protein solution	The protein samples can be prepared with 0.015% sodium azide.
	Eluted protein was not neutralized quickly enough	If the eluted protein is not neutralized immediately, degradation will occur. We strongly recommend adding Protein Neutralizer EF in order to lower the pH.

Related Products	Product #
ProteoSpin™ Endotoxin Removal Micro Kit	22800
ProteoLadder 100, Lyophilized	13500
ProteoLadder 100	12300

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.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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