

ProteoSpin™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Maxi Kit

Product Insert

Product # 22200

The ProteoSpin™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Mini Kit provides a fast and simple procedure for:

1. Concentrating small volumes of total protein solutions, for buffer exchange, and for removing different types of salts from protein samples.
2. The removal of SDS, Triton® X-100 and other detergents from total protein samples, including lysates.
3. The rapid removal of endotoxins from up to 4 mg of previously purified proteins or peptides

Detergents are extensively used to prepare protein samples; however, these detergents must often be removed prior to downstream analysis because of their undesirable effects. This kit can remove greater than 95% of detergents from total protein samples while maintaining high protein recovery. The kit is able to remove all types of detergents including ionic, non-ionic and zwitter ionic detergents. It is designed to remove detergents from protein solutions either in their free form or bound form, as when complexed with the protein. Detergents including SDS, Triton® X-100, CHAPS, NP-40 and Tween 20 can be removed using the kit, with protein recoveries of 90 – 95% for most proteins.

The ProteoSpin™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Maxi Kit is also highly efficient in removing many different salts commonly used in the laboratory including, but not limited to, MgCl₂, NaCl, KCl, CaCl₂, LiCl and CsCl. The simultaneous removal of salts while concentrating a dilute protein solution makes the kit a convenient method for preparing proteins before running many downstream applications such as SDS-PAGE, isoelectric focusing, X-ray crystallography, NMR spectroscopy, mass spectroscopy and other applications.

The ProteoSpin™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Maxi Kit is also designed for the rapid removal of endotoxins from up to 4 mg of previously purified proteins or peptides, with protein recoveries of > 95% being achieved. 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. This kit efficiently reduces endotoxin levels to ≤ 0.1 EU/μg of protein, using spin column chromatography based on Norgen's proprietary resin as the separation matrix. The purified protein samples can be used in a number of downstream applications including sequencing, cloning, and *in vitro* and *in vivo* introduction into cells and organisms for various purposes

The ProteoSpin™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Maxi Kit contains all the solutions and columns for the processing of 4 samples. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The kit has a shelf life of at least 1 year when stored as suggested.

Specifications

Kit Specifications	
Maximum Protein Input	4 mg
Minimum Protein Input	0.25 mg
Maximum Column Volume Input	18 mL
Molecular Weight of Recovered Proteins	No Molecular Weight cut-off
Final Endotoxin Levels	≤ 0.1 EU/μg protein
Protein Recovery	90-95 %
% Detergent Removal	90-95 %
Detergents that can be Removed	Including Triton® X-100, CHAPS, NP-40 and Tween 20
Elution Volume	2 - 4 mL
Time to Complete 10 Purifications	30 - 40 minutes

Kit Components

Component	Product # 22200 (4 Samples)
Binding Buffer J	8 mL
Binding Buffer N	20 mL
Wash Solution M	130 mL
Wash Solution CIP	60 mL
Wash Solution N	130 mL
Wash Solution NIP	60 mL
Elution Buffer G	20 mL
Protein Neutralizer EF	2 mL
Endotoxin Removal Solution	1.5 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. 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 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

- Swing Bucket centrifuge (capable of spinning the 50 mL conical tubes)
- pH indicator paper
- Micropipettors
- Isopropanol
- 100 mL sterile bottles
- Other elution buffers (optional)
- Sterile, deionized water or Milli-Q® water

Notes prior to use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 1,000 x g except where noted. Please check your microcentrifuge 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.
- 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 CIP** by adding 60 mL of isopropanol (to be provided by the user) to the supplied bottle containing **Wash Solution CIP**. This will give a final volume of 120 mL. The label on the bottle has a box that can be checked to indicate that isopropanol has been added.
- Prepare a working concentration of **Wash Solution NIP** by adding 60 mL of isopropanol (to be provided by the user) to the supplied bottle containing **Wash Solution NIP**. This will give a final volume of 120 mL. The label on the bottle has a box that can be checked to indicate that isopropanol has been added.
- Ensure that the maximum protein input does not exceed 4 mg. If the protein input exceeds this, the sample will need to be processed using more than 1 column.
- If the sample volume exceeds the maximum volume input (18 mL), the sample will need to be sequentially loaded to the same 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.
- Ensure that during each centrifugation step the cap is screwed loosely onto the column
- Ensure that all particulates in your sample have been removed by either filtration or centrifugation prior to starting the procedure
- ***The composition and the final molarity of the supplied Elution Buffer G is compatible with any proteomic downstream application. Consult Appendix A (Optional Elution Buffers) for a list of alternative elution solutions that have been tested with the kit.***
- ***All solutions supplied for the purpose of Endotoxin removal has been manufactured using Endotoxin-free water. DO NOT use any other from the supplied reagents for the purpose of Endotoxin removal***

Procedures

For protein concentration and detergent clean-up, the kit comes with solutions for processing both acidic and basic proteins. Two procedures, one for acidic proteins and another for basic proteins, are described below. Proteins with isoelectric points (pI) of less than 7 are by definition acidic proteins. However, for purposes of using the kit, the protocol for acidic proteins applies to any protein whose pI is less than 8.0. Proteins with pI higher than 8.0 are purified using the protocol for basic proteins. If the pI of the protein being purified is not known, the theoretical pI may be calculated using the web-based applications at http://us.expasy.org/tools/pi_tool.html. For the concentration and detergent clean-up of total proteins, please follow the acidic protocol. For endotoxin removal applications, only one procedure is outlined below since this procedure will concentrate total proteins and remove endotoxins simultaneously.

Protocol 1. Concentration of Total Protein and Detergent Clean-up (Acidic Protocol)

1. Sample Preparation

- a. Obtain protein sample. If particulates are present, clarify the sample through either filtration or centrifugation.
- b. Determine the pH and volume of the protein sample.
- c. Adjust the pH of the protein sample to **3.5 - 4** using **Binding Buffer J** and mix content well.
- d. Verify that the pH is between 3.5 - 4, and add more **Binding Buffer J** if necessary. **Binding Buffer N** can be used to re-adjust your pH.

Note 1: If the protein solution is already at the desired pH or lower, **Binding Buffer J** does not need to be added.

Note 2: In some concentrated protein samples, precipitation may occur with the addition of the **Binding Buffer J**. This precipitate includes proteins, and thus should not be discarded. The precipitate should be resuspended as much as possible, and loaded onto the column with the rest of the sample.

- e. Add one volume of isopropanol to the pH-adjusted solution and mix well.

2. Column Activation

- a. Obtain a spin column with its 50 mL conical collection tube. Remove lid.
- b. Apply 5 mL of **Wash Solution CIP (after the addition of Isopropanol)** to the column. Screw the cap back on **LOOSELY**.
- c. Centrifuge at 1,000 x g for two minutes.
Note: Start timing only after centrifuge has reached desired speed.
- d. Repeat steps **2b** and **2c** to complete the column activation step. Discard the flowthrough.

3. Protein Binding

- a. Apply the protein sample (from the Sample Preparation Step) onto the column and centrifuge for two minutes. The column can accommodate a maximum of 20 mL per spin.
- b. Discard the flowthrough. Reassemble the spin column with its collection tube.
Note: If desired, the flowthrough can be saved in a fresh tube for assessing your protein's binding efficiency.

- c. Depending on your sample volume, repeat steps **3a** and **3b** until the entire protein sample has been applied to the column.
- d. Discard any remaining flowthrough and reassemble the spin column with its collection tube.

4. Column Wash

- a. Apply 10 mL of **Wash Solution CIP (after the addition of Isopropanol)** to the column and centrifuge for two minutes.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Add 10 mL of **Wash Solution M** to the column and centrifuge for two minutes.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Inspect the column and ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin an additional two minutes to dry.

5. Protein Elution

- a. Add 350 μ L Protein Neutralizer to a fresh 50 mL elution tube.
- b. Transfer the spin column, with bound protein, into the 50 mL elution tube from step **6a**.

- c. Apply 4 mL of **Elution Buffer C** to the column and let stand at room temperature for 5 minutes. Centrifuge the column assembly for 2 minutes at 220 x g followed by 5 minutes at 2,000 x g

Protocol 2. Concentration of Total Protein From Samples Not Containing Detergent (Acidic Protocol)

1. Sample Preparation

- a. Obtain protein sample. If particulates are present, clarify the sample through either filtration or centrifugation.
- b. Determine the pH and volume of the protein sample.
- c. Adjust the pH of the protein sample to **3.5 - 4** using **Binding Buffer J** and mix content well.
- d. Verify that the pH is between 3.5 - 4, and add more **Binding Buffer J** if necessary. **Binding Buffer N** can be used to re-adjust your pH.

Note 1: If the protein solution is already at the desired pH or lower, **Binding Buffer J** does not need to be added.

Note 2: In some concentrated protein samples, precipitation may occur with the addition of the **Binding Buffer J**. This precipitate includes proteins, and thus should not be discarded. The precipitate should be resuspended as much as possible, and loaded onto the column with the rest of the sample.

2. 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. Screw the cap back on **LOOSELY**.
- c. Centrifuge at 1,000 x g for two minutes.

Note: Start timing only after centrifuge has reached desired speed.

- d. Repeat steps **2b** and **2c** to complete the column activation step. Discard the flowthrough.

3. Protein Binding

- a. Apply the protein sample (from the Sample Preparation Step) onto the column and centrifuge for two minutes. The column can accommodate a maximum of 20 mL per spin.
- b. Discard the flowthrough. Reassemble the spin column with its collection tube.

Note: If desired, the flowthrough can be saved in a fresh tube for assessing your protein's binding efficiency.

- c. Depending on your sample volume, repeat steps **3a** and **3b** until the entire protein sample has been applied to the column.
- d. Discard any remaining flowthrough and reassemble the spin column with its collection tube.

4. Column Wash

- a. Apply 10 mL of **Wash Solution M** to the column and centrifuge for two minutes.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Add 10 mL of **Wash Solution M** to the column and centrifuge for two minutes.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Inspect the column and ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin an additional two minutes to dry.

5. Protein Elution

- a. Add 350 μL Protein Neutralizer to a fresh 50 mL elution tube.
- b. Transfer the spin column, with bound protein, into the 50 mL elution tube from step 6a.
- c. Apply 4 mL of **Elution Buffer C** to the column and let stand at room temperature for 5 minutes. Centrifuge the column assembly for 2 minutes at 220 x g followed by 5 minutes at 2,000 x g

Protocol 3. Concentration of Basic Proteins and Detergent Clean-up (Basic Protocol)

1. Sample Preparation

- a. Obtain protein sample. If particulates are present, clarify the sample through either filtration or centrifugation.
- b. Determine the pH and volume of the protein sample.
- c. Adjust the pH of the protein sample to 7.0 using the **Binding Buffer N**. The amount of **Binding Buffer N** required will depend on the starting protein solution. If the starting protein solution is in water, then add one part of the **Binding Buffer N** to 50 parts of the protein solution. However, if the starting protein solution already contains a buffer, a greater volume of **Binding Buffer N** may be needed depending on the sample's buffer type and strength, as well as the type of protein. Table 1 below serves only as a guideline for the amount of **Binding Buffer N** to add for every milliliter of a protein solution in a 100 mM buffer to obtain pH 7.0. Please check the pH after mixing and add more **Binding Buffer N** if necessary to obtain the desired pH.

Table 1. pH Adjustment for Basic Proteins

Starting pH of Solution	Volume of Binding Buffer N per mL of protein solution (based on 100 mM buffered solution)
4	150 μL
5,6	80 μL
7	0 μL
8, 9, 10	60 μL
11, 12	80 μL

- d. Add one volume of isopropanol to the pH-adjusted solution and mix well.

2. Column Activation

- a. Obtain a spin column with its 50 mL conical collection tube. Remove lid.
- b. Apply 5 mL of **Wash Solution NIP (after the addition of Isopropanol)** to the column. Screw the cap back on **LOOSELY**.
- c. Centrifuge at 1,000 x g for two minutes.
Note: Start timing only after centrifuge has reached desired speed.
- d. Repeat steps 2b and 2c to complete the column activation step. Discard the flowthrough.

3. Protein Binding

- a. Apply the protein sample (from the Sample Preparation Step) onto the column and centrifuge for two minutes. The column can accommodate a maximum of 20 mL per spin.
- b. Discard the flowthrough. Reassemble the spin column with its collection tube.
Note: If desired, the flowthrough can be saved in a fresh tube for assessing your protein's binding efficiency.

- c. Depending on your sample volume, repeat steps **3a** and **3b** until the entire protein sample has been applied to the column.
- d. Discard any remaining flowthrough and reassemble the spin column with its collection tube.

4. Column Wash

- a. Apply 10 mL of **Wash Solution NIP (after the addition of Isopropanol)** to the column and centrifuge for two minutes.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Add 10 mL of **Wash Solution N** to the column and centrifuge for two minutes.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Inspect the column and ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin an additional two minutes to dry.

5. Protein Elution

- a. Add 350 μ L Protein Neutralizer, if desired (not required for the 1st elution), to a fresh 50 mL elution tube.
- b. Transfer the spin column, with bound protein, into the 50 mL elution tube from step **6a**.
- c. Apply 4 mL of **Elution Buffer C** to the column and let stand at room temperature for 5 minutes. Centrifuge the column assembly for 2 minutes at 220 x g followed by 5 minutes at 2,000 x g

Protocol 4. Concentration of Basic Proteins From Samples Not Containing Detergent (Basic Protocol)

1. Sample Preparation

- a. Obtain protein sample. If particulates are present, clarify the sample through either filtration or centrifugation.
- b. Determine the pH and volume of the protein sample.
- c. Adjust the pH of the protein sample to 7.0 using the **Binding Buffer N**. The amount of **Binding Buffer N** required will depend on the starting protein solution. If the starting protein solution is in water, then add one part of the **Binding Buffer N** to 50 parts of the protein solution. However, if the starting protein solution already contains a buffer, a greater volume of **Binding Buffer N** may be needed depending on the sample's buffer type and strength, as well as the type of protein. Table 2 below serves only as a guideline for the amount of **Binding Buffer N** to add for every milliliter of a protein solution in a 100 mM buffer to obtain pH 7.0. Please check the pH after mixing and add more **Binding Buffer N** if necessary to obtain the desired pH.

Table 2. pH Adjustment for Basic Proteins

Starting pH of Solution	Volume of Binding Buffer N per mL of protein solution (based on 100 mM buffered solution)
4	150 μ L
5, 6	80 μ L
7	0 μ L
8, 9, 10	60 μ L
11, 12	80 μ L

2. Column Activation

- a. Obtain a spin column with its 50 mL conical collection tube. Remove lid.
- b. Apply 5 mL of **Wash Solution N** to the column. Screw the cap back on **LOOSELY**.
- c. Centrifuge at 1,000 x g for two minutes.
Note: Start timing only after centrifuge has reached desired speed.
- d. Repeat steps **2b** and **2c** to complete the column activation step. Discard the flowthrough.

3. Protein Binding

- a. Apply the protein sample (from the Sample Preparation Step) onto the column and centrifuge for two minutes. The column can accommodate a maximum of 20 mL per spin.
- b. Discard the flowthrough. Reassemble the spin column with its collection tube.
Note: If desired, the flowthrough can be saved in a fresh tube for assessing your protein's binding efficiency.
- c. Depending on your sample volume, repeat steps **3a** and **3b** until the entire protein sample has been applied to the column.
- d. Discard any remaining flowthrough and reassemble the spin column with its collection tube.

4. Column Wash

- a. Apply 10 mL of **Wash Solution N** to the column and centrifuge for two minutes.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Add 10 mL of **Wash Solution N** to the column and centrifuge for two minutes.
- d. Inspect the column and ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin an additional two minutes to dry.

5. Protein Elution

- a. Add 350 μ L Protein Neutralizer, if desired (not required for the 1st elution), to a fresh 50 mL elution tube.
- b. Transfer the spin column, with bound protein, into the 50 mL elution tube from step **6a**.
- c. Apply 4 mL of **Elution Buffer C** to the column and let stand at room temperature for 5 minutes. Centrifuge the column assembly for 2 minutes at 220 x g followed by 5 minutes at 2,000 x g

Protocol 5. Procedure For Endotoxin Removal

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 μ L of **Binding Buffer J** for every milliliter 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 μ L 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 μ L 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 μ L 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 column and let stand at room temperature for 5 minutes. Centrifuge the column assembly for 2 minutes at 220 x g followed by 5 minutes at 2,000 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 μ L as well.

Appendix 1

Optional Elution Buffers

Proteins bound to Norgen's spin columns are eluted through pH-dependent mechanisms. The efficiency of protein elution depends on high pH above the pI of the protein to be purified. The pH of the elution buffer chosen must be at least one unit higher than the pI of the protein of interest. Solutions not provided with the ProteoSpin™ Detergent Clean-Up Maxi Kit may be utilized if they are more appropriate for your needs. The table below lists optional elution buffers and their observed efficiency when BSA is used as a test protein.

Elution Buffers	Approximate Protein Recovery
50 mM ammonium hydroxide (approximate pH 11)	70%
250 mM ammonium hydroxide (approximate pH 11)	70%
1 M ammonium hydroxide (approximate pH 11)	90%
1 M ethanolamine (approximate pH 9)	70-80%
50 mM sodium phosphate (approximate pH 12.5)	95%
500 mM sodium phosphate (approximate pH 12.5)	<70%
100 mM sodium borate (approximate pH 12.5)	95 -100%
1 M Tris (approximate pH 12.5)	95%

Troubleshooting Guide

A) For Protein Concentration and Detergent Clean-Up		
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 time.	Spin an additional minute or two to ensure the liquid has passed through the resin.
	Protein solution is too viscous.	Dilute the protein solution and adjust the pH to either 4.5 or 7 with the appropriate pH Binding Buffer. Highly viscous materials due to high protein concentrations can slow down flow rate significantly.
	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 severe clogging problems.
Poor peptide recovery	Protein solution is not completely dissolved.	Dissolve the sample in a larger amount of buffer. Solid, insoluble materials can cause clogging problems.
	Initial volume of sample applied to the column was too low.	Load at least 4 mL onto the column. This volume ensures that the entire bed is covered sufficiently.
	Incorrect procedure was used.	Ensure that the acidic protocol was used for acidic proteins and the basic protocol was used for basic proteins. It is known that when basic proteins are bound with the acidic protocol, elution is inefficient because the basic proteins are bound too tightly.
	Incorrect pH adjustment of sample.	Ensure that the pH of the starting protein sample is 4.5 for acidic proteins and 7.0 for basic proteins.
	Protein may have precipitated prior to loading onto the column.	If the pH of the protein solution is the same as the pI of the protein(s), precipitation may occur. In this case, adjust the pH of the sample to at least 1 pH unit lower than the pI of your protein.

Eluted protein is degraded	Eluted protein was not neutralized.	Add 300 μ L of Neutralizer to each 4 mL of eluted protein for all basic protein solutions. Acidic proteins tend not to need the neutralization for the first elution but require it for the second or third. Check the pH of the first elution if you are using an acidic protein.
	Proteases may be present.	Use protease inhibitors during all steps of the Sample Preparation.
	Bacterial contamination of protein solution.	Prepare the protein sample with 0.015% sodium azide. The elution buffer already contains sodium azide.
	Eluted protein was not neutralized quickly enough.	If eluted protein is not neutralized immediately, degradation will occur. We strongly recommend adding Neutralizer in order to lower pH.
B) For Endotoxin Removal		
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 μ M 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 μ L of Binding Buffer J is added for every 1 mL of protein processed. The initial protein volume must not exceed 18 mL.
B) For Endotoxin Removal		
Problem	Possible Cause	Solution and Explanation
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.
Eluted protein is degraded	Eluted protein was not neutralized	Add 140 μ L 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™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Mini 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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