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

Product Insert **Product # 22800**

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 200 µg 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 Mini Kit is also highly efficient in removing many different salts commonly used in the laboratory including, but not limited to, $MgCl_2$, NaCl, KCl, $CaCl_2$, 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 Mini Kit is also designed for the rapid removal of endotoxins from up to 200 µg 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 ≤ 0.01 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 Mini Kit contains all the solutions and columns for the processing of 25 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	200 μg
Maximum Column Volume Input	600 μL
Molecular Weight of Recovered Proteins	No Molecular Weight cut-off
Final Endotoxin Levels	≤ 0.01 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
Minimum Elution Volume	50 μL
Time to Complete 10 Purifications	20 minutes

Kit Components

Component	Product # 22800 (25 samples)
Binding Buffer J	8 mL
Binding Buffer N	4 mL
Wash Solution M	50 mL
Wash Solution CIP	20 mL
Wash Solution N	30 mL
Wash Solution NIP	20 mL
Elution Buffer G	6 mL
Protein Neutralizer EF	2 mL
Endotoxin Removal Solution	1.5 mL
Mini Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
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 from 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

- Benchtop microcentrifuge
- Micropipettors
- 1.5 mL microcentrifuge tubes
- Isopropanol
- Milli-Q® water
- Other elution buffers (optional)
- pH indicator paper
- Sterile, deionized water or Milli-Q® water

Notes prior to use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 5,200 x g (~8,000 RPM) 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.
- Ensure that no more than 200 µg of protein sample is used per column.
- Prepare a working concentration of **Wash Solution CIP** by adding 20 mL of isopropanol (to be provided by the user) to the supplied bottle containing **Wash Solution CIP**. This will give a final volume of 40 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 20 mL of isopropanol (to be provided by the user) to the supplied bottle containing **Wash Solution NIP**. This will give a final volume of 40 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 200 µg**.** 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 (600 µL), 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.
- ***The composition and the final molarity of the supplied Elution Buffer G and Protein Neutralizer EF are 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 have been manufactured using Endotoxin-free water. DO NOT use any of the other 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 Proteins 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 contents 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.** Assemble a spin column with a provided collection tube.
   - **b.** Apply 250 µL of **Wash Solution CIP (after the addition of Isopropanol)** to the column.
   - **c.** Centrifuge for one minute and discard the flowthrough.
   - **d.** Repeat steps **2b** and **2c** to complete the column activation step.

3. **Protein Binding**
   - **a.** Apply a maximum of 650 µL of protein solution (from the Sample Preparation step) onto the column and centrifuge for one minute.
   - **b.** Discard the flowthrough. Reassemble the spin column with its collection tube.
   - **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 250 µL of **Wash Solution CIP (after the addition of Isopropanol)** to the column and centrifuge for one minute.
   - **b.** Discard the flowthrough and reassemble the spin column with its collection tube.
   - **c.** Add 250 µL of **Wash Solution M** to the column and centrifuge for one minute.
   - **d.** Discard the flowthrough and reassemble the spin column with its collection tube.
   - **e.** Spin the column at **14,000 x *g* (~14,000 RPM)** for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. **Protein Elution**
   - **a.** Add 9.3 µL **Protein Neutralizer EF** to a provided 1.7 mL Elution tube.
   - **b.** Transfer the spin column from the Column Wash procedure into the Elution tube.
   - **c.** Apply 100 µL of **Elution Buffer G** to the column and let stand at room temperature for 2 minutes.
   - **d.** Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **6,700 x *g* (~10,000 RPM).** Note the volume eluted from the column.

Note: Approximately 95% of bound protein is recovered in the first elution. If desired, a second elution using 50 µL of **Elution Buffer G** may be carried out. This should be collected into a

different tube (to which 4.65 µL of **Protein Neutralizer EF** is pre-added) to prevent dilution of the first elution.

Protocol 2. Concentration of Total Proteins 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 contents 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.** Assemble a spin column with a provided collection tube.
   - **b.** Apply 250 µL of **Wash Solution M** to the column.
   - **c.** Centrifuge for one minute and discard the flowthrough.
   - **d.** Repeat steps **2b** and **2c** to complete the column activation step.

3. **Protein Binding**
   - **a.** Apply a maximum of 650 µL of protein solution (from the Sample Preparation step) onto the column and centrifuge for one minute.
   - **b.** Discard the flowthrough. Reassemble the spin column with its collection tube.
   - **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 250 µL of **Wash Solution M** to the column and centrifuge for one minute.
   - **b.** Discard the flowthrough and reassemble the spin column with its collection tube.
   - **c.** Add 250 µL of **Wash Solution M** to the column and centrifuge for one minute.
   - **d.** Discard the flowthrough and reassemble the spin column with its collection tube.
   - **e.** Spin the column at **14,000 x *g* (~14,000 RPM)** for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. **Protein Elution**
   - **a.** Add 9.3 µL **Protein Neutralizer EF** to a provided 1.7 mL Elution tube.
   - **b.** Transfer the spin column from the Column Wash procedure into the Elution tube.
   - **c.** Apply 100 µL of **Elution Buffer G** to the column and let stand at room temperature for 2 minutes.
   - **d.** Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **6,700 x *g* (~10,000 RPM).** Note the volume eluted from the column.

Note: Approximately 95% of bound protein is recovered in the first elution. If desired, a second elution using 50 µL of **Elution Buffer G** may be carried out. This should be collected into a different tube (to which 4.65 µL of **Protein Neutralizer EF** is pre-added) to prevent dilution of the first elution.

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
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.** Assemble a spin column with a provided collection tube.
- **b.** Apply 250 µL of **Wash Solution NIP (after the addition of isopropanol)** to the column.
- **c.** Centrifuge for one minute and discard the flowthrough.
- **d.** Repeat steps **2b** and **2c** to complete the column activation step.

3. Protein Binding

- **a.** Apply a maximum of 650 µL of protein solution (from the Sample Preparation step) onto the column and centrifuge for one minute.
- **b.** Discard the flowthrough. Reassemble the spin column with its collection tube.
- **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 250 µL of **Wash Solution NIP (after the addition of isopropanol)** to the column and centrifuge for one minute.
- **b.** Discard the flowthrough and reassemble the spin column with its collection tube.
- **c.** Add 250 µL of **Wash Solution N** to the column and centrifuge for one minute.
- **d.** Discard the flowthrough and reassemble the spin column with its collection tube.

e. Spin the column at **14,000 x *g* (~14,000 RPM)** for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. Protein Elution

- **a.** Add 9.3 µL **Protein Neutralizer EF** to a provided 1.7 mL Elution tube.
- **b.** Transfer the spin column from the Column Wash procedure into the Elution tube.
- **c.** Apply 100 µL of **Elution Buffer G** to the column and let stand at room temperature for 2 minutes.
- **d.** Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **6,700 x *g* (~10,000 RPM).** Note the volume eluted from the column.

Note: Approximately 95% of bound protein is recovered in the first elution. If desired, a second elution using 50 µL of **Elution Buffer G** may be carried out. This should be collected into a different tube (to which 4.65 µL of **Protein Neutralizer EF** is pre-added) to prevent dilution of the first elution.

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 µL
5, 6	80 µL
8, 9, 10	60 µL
11, 12	80 µL

2. Column Activation

- **a.** Assemble a spin column with a provided collection tube.
- **b.** Apply 250 µL of **Wash Solution N** to the column.
- **c.** Centrifuge for one minute and discard the flowthrough.
- **d.** Repeat steps **2b** and **2c** to complete the column activation step.

3. Protein Binding

- **a.** Apply a maximum of 650 µL of protein solution (from the Sample Preparation step) onto the column and centrifuge for one minute.
- **b.** Discard the flowthrough. Reassemble the spin column with its collection tube.
- **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 250 μL of **Wash Solution N** to the column and centrifuge for one minute.
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Add 250 μL of **Wash Solution N** to the column and centrifuge for one minute.
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Spin the column at **14,000 x *g* (~14,000 RPM)** for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. Protein Elution

a. Add 9.3 μL **Protein Neutralizer EF** to a provided 1.7 mL Elution tube.
b. Transfer the spin column from the Column Wash procedure into the Elution tube.
c. Apply 100 μL of **Elution Buffer G** to the column and let stand at room temperature for 2 minutes.
d. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **6,700 x *g* (~10,000 RPM)**.

Note: Approximately 95% of bound protein is recovered in the first elution. If desired, a second elution using 50 μL of **Elution Buffer G** may be carried out. This should be collected into a different tube (to which 4.65 μL of **Protein Neutralizer EF** is pre-added) to prevent dilution of the first elution.

Protocol 5. Procedure For **Endotoxin Removal**

1. Column Activation

a. Assemble a spin column with a provided collection tube.
b. Apply 500 μL of **Wash Solution M** to the column.
c. Centrifuge for 1 minute and discard the flowthrough.
d. Repeat steps **1b** and **1c** to complete the column activation step.

2. Sample Preparation

a. Obtain protein sample and measure the volume. Bring the volume up to 450 μL using sterile, deionized water or Milli-Q® water.
b. Add 20 μL of **Binding Buffer J** to the protein sample and mix well.
c. Verify that the pH is between **3.5 - 4**. Add more **Binding Buffer J** if necessary in 20 μL increments.
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 5 μL for every 500 μL 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 50 μL of isopropanol for every 500 μL of sample on top of the column).

2. Binding to Column

a. Place the column into a microcentrifuge and centrifuge for one minute.
b. Discard the flowthrough and reassemble the spin column with its collection tube.

3. Washing Bound Protein

a. Apply 500 µL of **Wash Solution M** to the column assembly and centrifuge for one minute.
b. Discard the flowthrough and reassemble the spin column with the collection tube.
c. Repeat steps **3a** and **3b**.
d. Spin the column at **14,000 x *g* (~14,000 RPM)** for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. Elution of Clean Protein

a. Add 9.3 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 50 µL of **Elution Buffer G** and let stand at room temperature for 2 minutes. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **6,700 x *g* (~10,000 RPM)**.
d. Without removing the assembly, add another 50 µL of **Elution Buffer G** and let stand at room temperature for 2 minutes. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **6,700 x *g* (~10,000 RPM)**.

Note: The majority of the input proteins will be recovered in the first elution. However, a second elution may be performed if desired. Steps 4a to 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 total elution volume can also be reduced to 50 µL if required (1 x 50 µL elution), but the amount of Protein Neutralizer EF EF must be reduced to 4.7 µL 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™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Mini 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 during the binding step	Centrifugation speed was too low	Check the centrifuge to ensure that it is capable of generating 5,200 x g (~8,000 RPM). Sufficient centrifugal force is required to move the liquid phase through the resin. Centrifugation speeds may be increased to 14,000 x *g* (~14,000 RPM), but this speed should not be exceeded.
	Inadequate spin time	Spin an additional two minutes to ensure that the liquid is able to flow completely 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 severe clogging problems.
	Protein solution is too viscous.	Dilute the protein solution and adjust the pH to either 3.5 - 4 or 7 with the appropriate Binding Buffer. Highly viscous materials due to high protein concentrations can slow down flow rate significantly.
	Protein solution is not completely dissolved.	Dissolve the sample in a larger amount of buffer. Solid, insoluble materials can cause clogging problems.
Poor protein/peptide recovery	Incorrect pH adjustment of protein sample.	It is important that the proper amount of the appropriate Binding Buffer is to be added to the protein sample in order to adjust the pH to either 3.5 - 4 or 7 prior to loading onto the column.
	Incorrect procedure was used.	Ensure that the acidic protocol was used for total proteins and the basic protocol was used for basic proteins.
Eluted protein is degraded	Eluted protein solution was not neutralized.	Add 9.3 μL of Neutralizer to each 100 μL of eluted total proteins in order to adjust the pH to neutral. Some proteins are sensitive to high pH, such as the elution buffer at pH 12.5.
	Eluted protein was not neutralized quickly enough.	If eluted proteins are not used immediately, degradation will occur. We strongly suggest adding Neutralizer in order to lower the pH.
	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.

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 to ensure that it is capable of generating 5,200 x g (~8,000 RPM). Sufficient centrifugal force is required to move the liquid phase through the resin. Centrifugation speeds may be increased to 14,000 x g (~14,000 RPM), but this speed should not be exceeded.
	Inadequate spin time	Spin for an additional minute 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 450 μL input, the sample can be split and processed using 2 columns.
	The appropriate amount of **Binding Buffer J** was not added	Ensure that 20μL of **Binding Buffer J** is added for every 500 μL of protein processed. The protein volume must not exceed 450 μL.

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 9.3 µL of Protein Neutralizer EF EF for every 100 µL 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 EF in order to lower the pH.

Related Products	**Product #**
ProteoSpin™ Total Protein Concentration, Detergent Clean-Up and Endotoxin Removal Maxi Kit	22200
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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