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ProteoSpin™ CBED (Concentration, Buffer Exchange and Desalting) Micro Kit Product Insert

Product # 10100, 10400

The ProteoSpin™ CBED Micro Kit provides a fast and simple procedure for concentrating small volumes of dilute protein solutions, for buffer exchange, and for removing different types of salts from protein samples. The kit is 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. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix.

The ProteoSpin™ CBED Micro Kit contains solutions for the processing of both acidic and basic protein samples. Each spin column is able to concentrate and desalt up to 50 μg of acidic or basic proteins. The kit has a shelf life of at least 1 year when stored as suggested.

Kit Components

Component	Product # 10100 (25 Samples)	Product # 10400 (50 Samples)
Column Equilibration and Wash Buffer (Acidic)	30 mL	60 mL
Column Activation and Wash Buffer (Basic)	30 mL	60 mL
pH Binding Buffer (Acidic)	3 mL	6 mL
pH Binding Buffer (Basic)	3 mL	6 mL
Elution Buffer	4 mL	8 mL
Neutralizer	1 mL	1 mL
Micro Spin Columns (assembled with collection tubes)	25	50
Elution tubes (1.7 mL)	30	60
Product Insert	1	1

Storage Conditions and Product Stability

For unopened solution containers, the reagents should remain stable for 12 months when stored at room temperature. Once opened, all solutions, except for the two binding buffers, should be stored at 4°C when not in use. The binding buffers should remain at room temperature with the lids tightly closed. Salt crystal formation may occur when stored at 4°C. If crystals are visible, bring the entire bottle to room temperature and mix gently to redissolve.

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.

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

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- pH indicator paper
- Micropipettors
- Other elution buffers (optional)

Procedure

The ProteoSpin™ CBED Micro Kit is designed for concentrating small volumes of dilute protein solutions, for buffer exchange, and for removing different types of salts from protein samples. The kit utilizes spin columns, which bind the protein of interest if it retains a net positive charge. Non-specifically bound materials such as salts are washed from the column and the specific protein is eluted into a small volume of elution buffer. The process results in an effective concentration and desalting of the protein. Each spin column is able to concentrate and desalt up to 50 μg of acidic or basic protein.

The ProteoSpin™ CBED Micro Kit comes with solutions for concentrating and desalting both acidic and basic proteins. Two procedures, one for acidic proteins and another for basic proteins, are described. 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*.

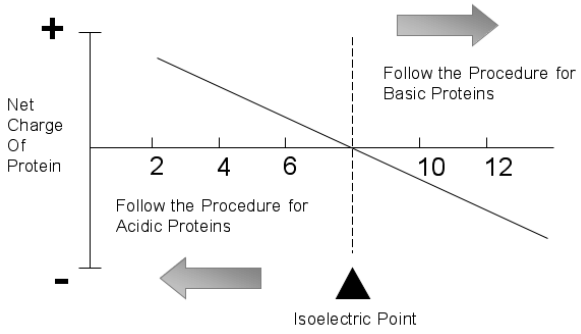

Figure 1. Choosing a procedure based on the isoelectric point (pI).

Protocol 1. CBED Protocol for Acidic Proteins

Proteins with an isoelectric point (pI) of less than 7 are by definition acidic proteins. However, for the purposes of using this kit, the Protocol for Acidic Proteins applies to any protein whose pI is less than 8.0

All centrifugation steps are carried out at 14,000 x g in a benchtop microcentrifuge. Performance of this kit is not affected by temperature, and thus the procedure may be performed at room temperature, 4°C, or on ice. The user must take discretionary measures, such as chilling samples in ice, to preserve biological activity.

Notes Before Use:

- Ensure that all particulates in your sample have been removed by either filtration or centrifugation prior to starting the procedure
- The column reservoir has a capacity of 1 mL; hence multiple centrifugations will be required for larger volumes

1. Sample Preparation

This step ensures that the protein solution is at the proper pH for column binding.

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 4.5 using the **pH Binding Buffer (Acidic)**. The amount of **pH Binding Buffer (Acidic)** required will depend on the starting protein solution. If the starting protein solution is in water, then add one part of the **pH Binding Buffer (Acidic)** to 50 parts of the protein solution. However, if the starting protein solution already contains a buffer, a greater volume of **pH Binding Buffer (Acidic)** 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 **pH Binding Buffer (Acidic)** to add for every milliliter of a protein solution in a 100 mM buffer to obtain pH 4.5. Please check the pH after mixing and add more **pH Binding Buffer (Acidic)** if necessary to obtain the desired pH.

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

Table 1. pH Adjustment for Acidic Proteins

Starting pH of Solution	Volume of pH Binding Buffer (Acidic) per mL of protein solution (based on 100 mM buffered solution)
5, 6, 7	20 µL
8	50 µL
9, 10, 11	80 µL
12	100 µL

d. Mix contents well, and measure the pH. Further adjust the pH if necessary.

e. Set aside until the Protein Binding step.

2. Column Activation

a. Open the cap on the pre-assembled spin column with its 2 mL collection tube.
b. Apply 250 µL of **Column Activation and Wash Buffer (Acidic)** to the column and close the cap.
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 1 mL 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.

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 250 µL of **Column Activation and Wash Buffer (Acidic)** to the column and centrifuge for one minute.
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Add another 250 µL of **Column Activation and Wash Buffer (Acidic)** to the column and centrifuge for one minute.
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 for an additional minute to dry.

5. Protein Elution

The supplied Elution Buffer consists of 50 mM sodium phosphate pH 12.5. Consult Appendix A (Optional Elution Buffers) for a list of alternative elution solutions that have been tested with the kit.

a. Add 5 µL Neutralizer to a provided 1.7 mL Elution tube.
b. Transfer the spin column from the Column Wash procedure into the Elution tube.
c. Apply 25 µL of Elution Buffer to the column and centrifuge for one minute to elute the bound protein.
d. Add another 25 µL of Elution Buffer and centrifuge for one minute into the same microcentrifuge tube.

Note: Approximately 95% of bound protein is recovered in the first two elutions. If desired, a third elution using 50 µL of Elution Buffer may be carried out. This should be collected into a different tube (to which 5 µL of Neutralizer is pre-added) to prevent dilution of the first two elutions.

Protein samples are now ready for downstream applications.

Protocol 2. CBED Protocol for Basic Proteins

Proteins with an isoelectric point (pI) of less than 7 are by definition acidic proteins. However, for the purposes of using this kit, the Protocol for Acidic Proteins applies to any protein whose pI is less than 8.0. Proteins with a pI higher than 8.0 are purified using the Protocol for Basic Proteins.

All centrifugation steps are carried out at 14,000 x g in a benchtop microcentrifuge. Performance of this kit is not affected by temperature, and thus the procedure may be performed at room temperature, 4°C, or on ice. The user must take discretionary measures, such as chilling samples in ice, to preserve biological activity.

Notes Before Use:

- Ensure that all particulates in your sample have been removed by either filtration or centrifugation prior to starting the procedure
- The column reservoir has a capacity of 1 mL; hence multiple centrifugations will be required for larger volumes

1. Sample Preparation

This step ensures that the protein solution is at the proper pH for column binding.

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 **pH Binding Buffer (Basic)**. The amount of **pH Binding Buffer (Basic)** required will depend on the starting protein solution. If the starting protein solution is in water, then add one part of the **pH Binding Buffer (Basic)** to 50 parts of the protein solution. However, if the starting protein solution already contains a buffer, a greater volume of **pH Binding Buffer (Basic)** 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 **pH Binding Buffer (Basic)** 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 **pH Binding Buffer (Basic)** if necessary to obtain the desired pH.

Table 2. pH Adjustment for Basic Proteins

Starting pH of Solution	Volume of pH Binding Buffer (Basic) 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. Check the pH after mixing and add more pH Binding Buffer (Basic) if necessary to return the solution to a pH of 7.0.
e. Set aside until the Protein Binding step.

2. Column Activation

a. Open the cap on the pre-assembled spin column with its 2 mL collection tube.
b. Apply 250 μL of **Column Activation and Wash Buffer (Basic)** to the column and close the cap.
b. Centrifuge for one minute and discard the flowthrough.
c. Repeat steps **2b** and **2c** to complete the column activation step.

3. Protein Binding

a. Apply a maximum of 1 mL 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.

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 250 μL of **Column Activation and Wash Buffer (Basic)** to the column and centrifuge for one minute.
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Add another 250 μL of **Column Activation and Wash Buffer (Basic)** to the column and centrifuge for one minute.
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 for an additional minute to dry.

5. Protein Elution

The supplied Elution Buffer consists of 50 mM sodium phosphate pH 12.5. Consult Appendix A (Optional Elution Buffers) for a list of alternative elution solutions that have been tested with the kit.

- **a.** Add 5 μL Neutralizer to a provided 1.7 mL Elution tube.
- **b.** Transfer the spin column from the Column Wash procedure into the Elution tube.
- **c.** Apply 25 μL of Elution Buffer to the column and centrifuge for one minute to elute the bound protein.
- **d.** Add another 25 μL of Elution Buffer and centrifuge for one minute into the same microcentrifuge tube.

Note: Approximately 95% of bound protein is recovered in the first two elutions. If desired, a third elution using 50 μL of Elution Buffer may be carried out. This should be collected into a different tube (to which 5 μL of Neutralizer is pre-added) to prevent dilution of the first two elutions.

Protein samples are now ready for downstream applications.

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 14,000 x g. Sufficient centrifugal force is required to push the liquid through the column.
	Inadequate spin time.	Spin an additional minute to ensure that the liquid is able to flow completely through the column.
	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.
	Protein solution is not completely dissolved.	Dissolve the sample in a larger amount of buffer. Solid, insoluble materials can cause clogging problems.
Poor peptide recovery	Initial volume of sample applied to the column was too low.	Load at least 100 μL 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 5 μL of Neutralizer to each 50 μL of eluted protein in order to adjust the pH to neutral. Some proteins are sensitive to high pH, such as the elution buffer at pH 12.5.
	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 the pH.

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™ CBED Micro 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%

Related Products	Product #
ProteoSpin™ Detergent Clean-up Micro Kit (25 samples)	10200
ProteoSpin™ Detergent Clean-up Maxi Kit (4 samples)	17100
ProteoSpin™ CBED Maxi Kit (4 samples)	17000

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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