

ProteoSpin™ Urine Protein Concentration Micro Kit
Product # 17400**Product Insert**

Urine protein analysis can be used for the identification of potential biomarkers in urine, and to diagnose and/or monitor renal and other diseases. The ProteoSpin™ Urine Protein Concentration Micro Kit provides a fast and simple procedure for concentrating dilute solutions of urine proteins from small volumes of urine. Purification is based on spin column chromatography using Norgen's proprietary resin as an ion exchanger. Urine proteins will bind to the column, while non-specifically bound materials such as salts are easily removed from the sample. The simultaneous removal of salts while concentrating a dilute urine protein solution makes the kit a convenient method for preparing proteins before running downstream proteomic applications including SDS-PAGE, 2D gels, whole protein mass spectrometry, and protein microarrays.

The ProteoSpin™ Urine Protein Concentration Micro Kit contains sufficient materials for 25 preparations. Each spin column is able to concentrate and desalt up to 200 µg of urine proteins. Preparation time for 12 samples is only 20 minutes. The kit has a shelf life of at least 1 year when stored as suggested.

Kit Components

Component	Product # 17400 (25 samples)
Column Activation and Wash Buffer	60 mL
pH Binding Buffer	2 mL
Stabilizer	1 mL
Elution Buffer	6 mL
Neutralizer	1 mL
Micro Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
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Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. Once opened, the solutions should be stored at 4°C. 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.norgenbiotech.com.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 1.5 mL microcentrifuge tubes
- Protease Inhibitor Cocktail (optional)

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 3,300 x g (~7,000 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.

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.

1. Urine Sample Collection

- a. Collect 1 mL of urine sample into a sterile 1.5 mL microcentrifuge tube that contains 10 μ L of Stabilizer.

Note: If the urine sample is not going to be processed within a day of collection, protease inhibitors must be added. We recommend that Sigma's Protease Inhibitor Cocktail is used (Product Number P2714). This product contains a mixture of protease inhibitors known to be very effective with our kit. The cocktail includes AEBSF, EDTA, Bestatin, E-64, Leupeptin and Aprotinin. Add 10 μ L of this cocktail to the 1 mL sample of urine, as per manufacturer's instructions.

2. pH Adjustment of Urine Sample

The most critical step in urine protein sample preparation is the proper pH adjustment of the solution to be applied to the column. Depending on the person's acid-base status, the pH of the urine sample may range from 4.5 to 8. The pH of the urine must be adjusted to the binding pH of 3.5 in order to concentrate the urine proteins.

- a. Add 40 μ L of pH Binding Buffer to the 1 mL urine sample prepared above.
- b. Mix contents well.
- c. Verify that the pH is 3.5, and add more pH Binding Buffer if necessary.

Note: In some concentrated urine samples, precipitation may occur with the addition of the pH Binding Buffer. This precipitate includes urine 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.

3. Column Activation

- a. Assemble a spin column with a provided collection tube. Open the cap on the column.
- b. Add 500 μL of Column Activation and Wash Buffer to the column and close the cap.
- c. Centrifuge for two minutes at 3,300 x g (~7,000 RPM).
- d. Repeat steps **3b** and **3c** to complete the column activation step.

4. Protein Binding

- a. Apply up to 650 μL of the pH-adjusted urine sample onto the column, and centrifuge for two minutes. Inspect the column to ensure that the entire sample has passed through into the collection tube. If necessary, spin for an additional three minutes.

Note: If the sample still has not passed into the collection tube after five minutes, the speed may be increased to 6,700 x g (~10,000 RPM) and the column spun for another two minutes.

- b. Discard the flowthrough. Reassemble the spin column with its collection tube.
- c. Repeat steps **4a** and **4b** until the entire protein sample has been loaded onto the column.

Note: You can save the flowthrough in a fresh tube for assessing your protein's binding efficiency.

5. Column Wash

- a. Apply 500 μL of Column Activation and Wash Buffer to the column and centrifuge for two minutes.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Add another 500 μL of Column Activation and Wash Buffer to the column and centrifuge for two minutes.
- d. Inspect the column to 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.

6. Protein Elution and pH Adjustment

The supplied Elution Buffer consists of 10 mM sodium phosphate pH 12.5.

- a. Add 9.3 μL of Neutralizer to a fresh 1.7 mL Elution Tube.
- b. Transfer the spin column from the Column Wash procedure into the Elution Tube.
- c. Apply 100 μL of the Elution Buffer to the column and centrifuge for two minutes to elute bound proteins.

Note: Approximately 90% of bound protein is recovered in the first elution. If desired, a second elution using 100 μL of Elution Buffer may be carried out. This should be collected into a different tube (to which 9.3 μL of Neutralizer is pre-added) to prevent dilution of the first elution.

Urine proteins 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 to ensure that it is capable of generating 3,300 x g. Sufficient centrifugal force is required to move the liquid phase through the resin. Centrifugation speeds may be increased to 6,700 x g, 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.
Poor protein recovery	Incorrect pH adjustment of urine sample.	Depending on a person's acid-base status, the starting pH of the urine may range from 4.5 to 8. Therefore, it is important that the proper amount of pH Binding Buffer be added to the urine sample in order to adjust the pH to 3.5 prior to loading onto the column.
	Initial volume of sample applied to column was too low.	Ensure that 1 mL of the pH-adjusted urine sample is loaded onto the column in order to capture a large portion of the proteins present in the sample.
Eluted protein is degraded	Eluted protein solution was not neutralized.	Add 9.3 μ L of Neutralizer to each 100 μ L 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
	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.	Ensure that Protease Inhibitor Cocktail was used during the collection of the urine sample.
	Bacterial contamination of the protein solution.	Ensure that the Stabilizer was used during the collection of the urine sample.

Related Products	Product #
ProteoSpin™ Urine Protein Concentration 96-Well Kit	23100
ProteoSpin™ Urine Protein Concentration Maxi Kit	21600
Urine DNA Isolation Kit	18100

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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