

ProteoSpin™ Urine Protein Concentration Midi Kit
Product # 52300**Product Insert**

The ProteoSpin™ Urine Protein Concentration Midi Kit provides a fast and simple procedure for concentrating dilute solutions of urine proteins from 1 to 5 mL inputs of urine. 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 purified urine proteins are both concentrated and free of salts, making this kit a convenient method for preparing proteins before running downstream proteomic applications including SDS-PAGE, 2D gels, whole protein mass spectrometry, and protein microarrays.

Norgen's Purification Technology.

Purification is based on spin column chromatography using Norgen's proprietary resin as an ion exchanger. The resin has a poor affinity for monovalent and divalent cations, also making it an effective resin for the removal of salts. Thus in this case, urine proteins are preferentially purified from all other urine components including salts and other wastes. The process involves first collecting the urine sample and adjusting the pH to 3.5 using the provided Binding Buffer A (please see flow chart on page 3). By adjusting the pH to 3.5, the urine proteins present will be able to bind to the resin. The midi spin column is then activated, and the pH-adjusted urine sample is applied to the column. The urine proteins present will bind to the column based on their net charge, while the salts and other wastes will be removed in the flowthrough. The column with the bound proteins is then washed twice with the provided Wash Solution C, in order to remove any remaining impurities. The purified urine proteins are then eluted in a small volume of the provided Elution Buffer C. The concentrated and salt-free urine proteins can then be used in a number of downstream applications.

Specifications

Kit Specifications	
Minimum Urine Volume Input	1 mL
Maximum Urine Volume Input	5 mL
Time to Process 10 Samples	30 minutes
Minimum Elution Volume	0.2 mL

Advantages

- Fast and easy processing using a rapid spin-column format
- Broad size range of proteins can be processed – no molecular weight cutoff
- Concentrate and desalt urine proteins in 30 minutes

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. Once opened, the solution should be stored at 4°C. All the reagents should remain stable for at least 1 year in their unopened containers.

Kit Components

Component	Product # 52300 (10 samples)
Wash Solution C	60 mL
Binding Buffer A	4 mL
Elution Buffer C	30 mL
Protein Neutralizer	4 mL
Midi Spin Columns (assembled with collection tubes)	10
Midi Elution Tubes	10
Product Insert	1

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 2,000 x g
- Micropipettors
- 15 mL conical tubes

Procedure

All centrifugation steps are carried out in a benchtop centrifuge at 1,000 x g except where noted.

Please check your centrifuge specifications to ensure that it is capable of the proper speeds.

The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

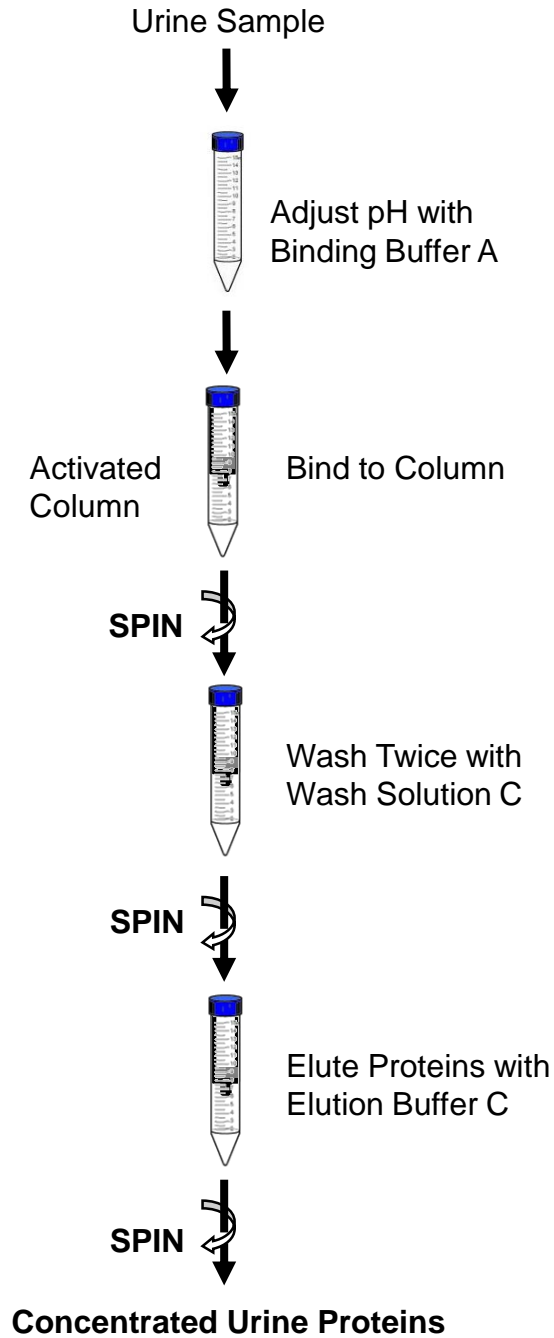
where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

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.
- We recommend the use of **Norgen's Urine Preservative** when collecting urine samples, which is designed for the preservation of nucleic acids and proteins in fresh urine samples at ambient temperatures. The components of the Urine Preservative allow samples to be stored for over 2 years at room temperature with no detected degradation of urine DNA, RNA or proteins. Norgen's Urine Preservative is available in 2 convenient formats: in a liquid format in Norgen's Urine Preservative Single Dose Ampules, as well as in a dried format in Norgen's Urine Collection and Preservation Tubes. Please see the Related Products table below.

Flow Chart

Procedure for Concentrating Urine Proteins using the ProteoSpin™ Urine Protein Concentration Midi Kit



1. 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 Binding Buffer A for every 1 mL sample of urine to be processed. Urine inputs from 1 mL to 5 mL can be processed using this kit.
- b. Mix contents well.

Note: In some concentrated urine samples, precipitation may occur with the addition of the Binding Buffer A. 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.

- c. Verify that the pH is at 3.5. Add more Binding Buffer A if necessary.

2. Column Activation

- a. Unscrew the cap on the pre-assembled spin column with its 15 mL collection tube.
- b. Add 1 mL of Wash Solution C to the column and cap very loosely.
- c. Centrifuge for three minutes at 1,000 x g.
- d. Repeat steps **2b** and **2c** to complete the column activation step. Discard the flowthrough.

3. Protein Binding

- a. Apply the entire pH-adjusted urine sample onto the column, and centrifuge for five 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 eight minutes, the speed may be increased to 2,000 x g and the column spun for another three minutes.

- b. Discard the flowthrough. Reassemble the spin column with its collection tube.

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

4. Column Wash

- a. Apply 1 mL of Wash Solution C to the column and centrifuge for three minutes.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Add another 1 mL of Wash Solution C to the column and centrifuge for five minutes to ensure complete dryness.
- 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.

5. Protein Elution and pH Adjustment

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

Note: *For full recovery of the concentrated urinary proteins, the volume of Elution Buffer and the volume of Neutralizer to add for pH adjustment of the eluted proteins will vary depending on the initial sample volume. The procedure below is outlined for the elution and pH adjustment of urinary proteins concentrated from 1mL sample urine. If a different urine sample volume was used please refer to Table 1 below*

- a. Add 18.6 μ L of Protein Neutralizer to a fresh 15 mL Elution Tube.

- b. Transfer the spin column from the Column Wash procedure into the Elution Tube.
- c. Apply 100 μ L of the Elution Buffer C to the column and centrifuge for 2 minutes to elute bound proteins.
- d. Repeat Step 5C one more time to have a final elution volume of 200 μ L

Table 1. Elution Buffer C and Protein Neutralizer Volumes to Use According to the Initial Urine Sample Volume

Urine Sample Volume	Protein Neutralizer (Step 5a)	Elution Buffer C (5c)	Elution Buffer C (5d)
1mL	18.6 μ L	100 μ L	100 μ L
2mL	37.2 μ L	200 μ L	200 μ L
3mL	55.8 μ L	300 μ L	300 μ L
4mL	74.4 μ L	400 μ L	400 μ L
5mL	93 μ L	500 μ L	500 μ L

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 1,000 x g. Sufficient centrifugal force is required to move the liquid phase through the resin. Centrifugation speeds may be increased to 2,000 x g, but this speed should not be exceeded.
	Inadequate spin time.	Spin an additional three 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 Binding Buffer A be added to the urine sample in order to adjust the pH to 3.5 prior to loading onto the column. With such large volumes, it is important to verify this and if necessary, add more Binding Buffer A to adjust the sample to pH 3.5.
	Initial volume of sample applied to column was too low.	Up to 5 mL of urine can be processed using this kit in order to capture a large portion of the proteins present in the sample.
Eluted protein is degraded	Eluted protein solution was not neutralized.	Ensure that Protein Neutralizer is added to the eluted protein in order to adjust the pH to neutral, as outlined in Table 1 above. 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 Protein Neutralizer in order to lower the pH.

Related Products	Product #
ProteoSpin™ Urine Protein Concentration 96-Well Kit	23100
ProteoSpin™ Urine Protein Concentration Micro Kit	17300
Urine DNA Isolation Kit	18100
Urine Collection and Preservation Tubes (50 cc) – 1 tube	18111
Urine Collection and Preservation Tubes (50 cc) – 50 tubes	18113
Urine Collection and Preservation Tubes (15 cc) – 1 tube	18120
Urine Collection and Preservation Tubes (15 cc) – 50 tubes	18122
Urine Collection and Preservation Tubes (5 cc) – 1 tube	18116
Urine Collection and Preservation Tubes (5 cc) – 50 tubes	18118
Urine Preservative Single Dose – 1 tube	18124
Urine Preservative Single Dose – 50 tubes	18126

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

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
 Phone: (905) 227-8848
 Fax: (905) 227-1061
 Toll Free in North America: 1-866-667-4362