NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

ProteoSpin™ On-Column Proteolytic Digestion Kit Product Insert

Product # 17500

The ProteoSpin™ On-Column Proteolytic Digestion Kit provides a fast and simple procedure for the generation of purified peptides. The kit is highly efficient in the enzymatic digestion of simple and complex protein samples using trypsin, and the subsequent purification of the resulting peptides. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Binding occurs if the protein or proteins of interest retain a net positive charge at the binding pH. Non-specifically bound materials such as salts are washed from the column and the bound protein and enzyme remain. At the binding pH, the activity of trypsin is inhibited. The trypsin is then activated and the digestion proceeds for approximately 2 hours. The peptides are then eluted into a small volume. The peptides generated are complete, with no additional artifacts being detected in mass spectrometry. The simultaneous protein digestion and volumetric concentration of the purified peptides makes the kit a convenient method for preparing peptides to be analyzed by many downstream applications such as SDS-PAGE and 2D gel electrophoresis, MALDI-TOF, LC/MS and LC/MS/MS.

The ProteoSpin™ On-Column Proteolytic Digestion Kit contains sufficient materials for 25 preparations. Each spin column is able to process, digest and purify samples containing up to 15 μg of protein. The kit has a shelf life of at least 1 year when stored as suggested.

Kit Components

Component	Product # 17500 (25 samples)
Wash Solution C	30 mL
Binding Buffer A	4 mL
Column Activation Buffer	3 mL
Micro 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. Once opened, the solution should be stored at 4°C. 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 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

- Benchtop microcentrifuge
- pH indicator paper
- Micropipettors
- Trypsin (sequencing grade)
- 37°C incubator
- 60°C incubator (optional)
- DTT (optional)
- Iodoacetamide (optional)

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature.

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.
- It is recommended that a minimum of 2 μg and a maximum of 15 μg of protein be bound to each column for digestion.
- For complex protein solutions such as urine, the recommended amount of protein to be used for digestion is 2 - 10 μg.
- The column reservoir has a capacity of 650 μL; hence multiple centrifugations will be required for larger volumes

1. Modification of Protein (Optional)

Note: This section describes the preferred method for reduction and alkylation of protein samples for use with the ProteoSpin™ On-Column Proteolytic Digestion Kit. This section is optional and is performed at the user's discretion.

a. Prepare a fresh 100 mM DTT solution in Milli-Q® water.
b. Add the DTT solution to the protein sample to a final concentration of 5 mM DTT. Incubate the sample at 60°C for 10 minutes.
c. While the protein is incubating, prepare a fresh solution of 1M iodoacetamide in Milli-Q® water. The iodoacteamide is light sensitive, thus ensure that it is stored properly.
d. Once the protein tube has cooled, add the 1M iodoacteamide to the mixture to a final concentration of 100 mM iodoacetamide. Incubate the sample at room temperature for 30 minutes in the dark.
e. Proceed with the Sample Preparation step.

2. 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 of the protein sample.
c. Adjust the pH of the protein sample to 3.5 using the **Binding Buffer A**. The amount of **Binding Buffer A** required will depend on the starting protein solution. If the starting protein solution is in water, then add one part of the **Binding Buffer A** to 50 parts of the protein solution. However, if the starting protein solution already contains a buffer, a greater volume of **Binding Buffer A** may be needed depending on the sample's buffer type and strength, as well as the type of protein. Please check the pH after mixing and add more **Binding Buffer A** if necessary to obtain the desired pH.

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

d. Once the pH has been adjusted to 3.5, add the trypsin to the protein sample. The trypsin should be added at a ratio of between 1:10 and 1:50 trypsin to protein. For example, if the sample contains 15 μg of protein, add between 0.3 μg and 1.5 μg of trypsin.
e. Set aside until the Protein Binding step.

3. Column Equilibration

a. Assemble a spin column with a provided collection tube. Open the cap on the column.
b. Add 250 μL of **Wash Solution C** to the column and close the cap.
c. Centrifuge at 6,700 x g (~ 10,000 RPM) for one minute.
d. Repeat steps **3b** and **3c** to complete the column equilibration step. Discard the flowthrough.

4. Protein Binding

a. Apply a maximum of 650 μL of protein sample (from the Sample Preparation Step) onto the column and centrifuge at 6,700 x g (~10,000 RPM) 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. The protein content of the flowthrough can be determined and compared with the initial input amount.

c. Depending on your sample volume, repeat steps **4a** and **4b** 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.

5. Column Wash

a. Apply 250 μL of **Wash Solution C** to the column and centrifuge at 6,700 x g (~10,000 RPM) for one minute.
b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Add another 250 μL of **Wash Solution C** to the column and centrifuge at 6,700 x g (~10,000 RPM) for one minute.
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. Enzyme Activation and Digestion

During this step, the bound trypsin will become active in the presence of 50 mM ammonium bicarbonate pH 7.7 (**Column Activation Buffer**).

a. Transfer the column to a fresh elution tube (supplied). Add 50 μL of **Column Activation Buffer** to the column and centrifuge at 200 x g (~2,000 RPM) for two minutes to activate the enzyme.
b. There will be a small amount of liquid (about 25 μL) in the elution tube after centrifugation. Transfer this liquid back onto the column.
c. Place the column at 37°C for 2 hours in order to allow for digestion.

Note: Digestion time can vary from 1-2 hours. Optimal digestion has been achieved in 2 hours using the enzyme conditions in Section 2.

7. Peptide Recovery

a. After the digestion has proceeded for the desired amount of time, centrifuge the column at 6,700 x g (~10,000 RPM) for one minute to elute the purified peptides.
b. If a greater volume of peptides are required for downstream applications, an additional volume of the **Column Activation Buffer** may be added to the column, and the column centrifuged again at 6,700 x g for one minute. This sample will be very dilute and should be kept separate from the first elution.

Peptide samples are now ready for downstream applications.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Protein solution does not flow through the column	Too much protein was loaded onto the column.	We recommend that between 2 μg and 15 μg of protein is bound to the column for digestion. For complex protein solutions, we recommend between 2 μg and 10 μg of protein be bound to the column.
	Centrifugation speed was too low.	Check the centrifuge and ensure that it is capable of generating 6,700 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 3.5 with the Binding Buffer A. 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 pH adjustment of sample.	Ensure that the pH of the starting protein sample is 3.5. This is adjusted with the Binding Buffer A.
	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, add more Binding Buffer A to lower the pH of the sample even further.

Related Products	Product #
ProteoSpin™ Total Protein Concentration and Detergent Clean-Up Mini Kit	23350
ProteoSpin™ Total Protein Concentration and Detergent Clean-Up Maxi Kit	17150

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

