

ProteoSpin[™] On-Column Proteolytic Digestion Kit

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.

Kit Specifications					
Maximum Protein Input	15 μg	Minimum Elution Volume	30 μL		
Minimum Protein Input	2 µg	Time to Process 10 Samples	20 minutes (Plus a 1 hour incu- bation)		

ProteoSpin™ On-Column Proteolytic Digestion Kit Benefits			
Fast method for generating peptides	Compared to traditional methods of peptide generation and purifi- cation, the ProteoSpin™ On-Column Proteolytic Digestion kit is very rapid.		
Does not generate artifacts	Peptide generation is complete, with no generation of additional artifacts being detected in mass spectrometry.		
Digestion and purification in a single column	The use of multiple columns and tubes for digestion and purification is avoided; lower cost and increased convenience for user.		
Suitable for downstream appli- cations	 Final elution suitable for: Mass spectrometry (LC/MS and LC/MS/MS) SDS-PAGE MALDI-TOF 		



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Figure 1. Generation of Tryptic BSA Peptides without the Generation of Artifacts. MALDI-TOF spectra of the peptides that resulted after BSA was digested on-column with trypsin using the ProteoSpin[™] On-Column Proteolytic Digestion Kit (Panel A) or digested in a typical liquid environment (Panel B). For both digestions, 15 µg of BSA was used as the input and 0.3 µg of Trypsin was used for the digestion. For on-column digestion, the 15 µg of BSA was loaded onto the column with the trypsin, the BSA was washed, then the on-column digestion proceeded and the resulting peptides were eluted. By comparing the spectra, it can be seen that the ProteoSpin[™] kit (Panel A) resulted in the generation of the same peptide profile in MALDI-TOF as the liquid digestion did (Panel B). Furthermore, no artifacts were found to arise from the use of the column, and all the same peaks could be detected in both spectra.

ProteoSpin™ On-Column Proteolytic Digestion Kit Contents

1. Wash Solution C

- 2. Binding Buffer A
- 3. Column Activation Buffer
- 4. Micro Spin Columns
- 5. Collection Tubes
- 6. Elution tubes (1.7 mL)
- 7. Product Insert

Storage Conditions

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

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)

Shipping Conditions

The ProteoSpin[™] On-Column Proteolytic Digestion Kit is shipped at room temperature.

Cat #	Description	Quantity
17500	ProteoSpin™ On-Column Proteolytic Digestion Kit	25 preps

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