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ProductInformation

Trypsin Spin Columns, Proteomics Grade
Immobilized Trypsin in a Spin Column Format

Product Number **TT0010**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Trypsin is routinely used in proteomics for peptide mapping and protein sequence work due to its highly specific cleavage resulting in a limited number of tryptic peptides.[1-5] Trypsin is a pancreatic serine endoprotease (EC 3.4.21.4 and CAS RN 9002-07-7) which hydrolyzes peptide bonds specifically at the carboxyl side of arginine and lysine residues. The rate of hydrolysis is slower if an acidic residue is located on either side of the cleavage site and cleavage may not occur if a proline residue is positioned on the carboxyl side.[1-5] The enzyme also exhibits esterase and amidase activities.[1] Trypsin has an average molecular weight of 23.29 kDa and optimal pH for activity near 8.0.[1]

Typical in-solution protein digestions require incubation periods of 4 hours to overnight at 37 °C. Trypsin autolytic fragments are commonly observed in these digests. By immobilizing the trypsin at a high density on a solid support and using it in a spin column format, the digestion time can be reduced to 15 minutes and little or no autolytic fragments are detected in the resultant peptide mixture. The solid support is spherical, 20 µm silica with 1,000 Å pore size, which has been chemically modified to minimize non-specific adsorption. The trypsin is highly purified from porcine pancreas and has been treated with TPCK to ensure low chymotryptic activity. The spin column format is optimal for rapid protein digestions in small volumes (100 µl or less).

Components

- Trypsin Spin Columns (Product Code H1914) 10 each
  50% acidic, glycerol suspension containing 75 mg (~150 µl) of solid support
- Collection Tubes – 2 ml capacity (Product Code T5449) 2 x 10 each
- Enzyme Reaction Buffer (Product Code E0530) 25 ml
  powder to prepare 25 ml of 100 mM ammonium bicarbonate

Equipment and Reagents Required But Not Provided

- Ultrapure water (18 MΩ×cm or equivalent)
- Protein Extraction Reagent Type 4 (Product Code C0356)
- ProteoPrep® Reduction and Alkylation Kit (Product Code PROTRA)
- Microcentrifuge with adjustable speeds

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Enzyme Reaction Buffer – Add 25 ml of ultrapure water to the bottle and mix until fully dissolved. This results in a 100 mM ammonium bicarbonate solution.

Storage/Stability

The kit ships on wet ice and storage at 2–8 °C is recommended. The columns, as supplied, are stable for at least one year at 2–8 °C. The columns should be used the same day they are washed and equilibrated. The prepared Enzyme Reaction Buffer is stable for at least one month at 2–8 °C.

Procedure

Denaturant Selection

Digestion efficiency can be enhanced or changed by the selection of the denaturant solution used in sample preparation. The choice of the denaturant solution is often determined by the subsequent downstream application(s). This procedure uses Protein Extraction Reagent 4 (Product Code C0356, a mixture of urea, thiourea, and C_7BzO detergent) as the denaturant and its use resulted in the complete digestion of all proteins tested.

Conversely, 8 M urea, 6 M guanidine HCl, and/or the inclusion of various organic solvents, such as acetonitrile, resulted in different digestion efficiencies for some proteins. The ProteoPrep Detergent Sample Kit (Product Code PROTDT) contains 10 non-ionic and zwitterionic detergents suitable for protein solubilization. One or more of these detergents may be incorporated into the denaturant solution at empirically determined optimal concentrations.

Trypsin Spin Column Wash and Equilibration

1. Column Wash Buffer Preparation

The Column Wash Buffer is prepared by diluting Protein Extraction Reagent 4 (Product Code C0356) 4-fold with the prepared Enzyme Reaction Buffer – Dilute 1 ml of reconstituted Protein Extraction Reagent 4 with 3 ml of Enzyme Reaction Buffer.

Note: When other denaturant solutions are used, the Column Wash Buffer is prepared with a 4-fold dilution of the denaturant solution with Enzyme Reaction Buffer. If 8 M urea is used as the denaturant, then the Column Wash Buffer would contain 2 M urea. For protein samples denatured by heat, a 2 M Urea Column Wash Buffer may be used to prepare the column.

2. Initial Column Preparation Instructions

The trypsin-silica resin is packed in the columns as a 50% suspension in acidic glycerol. If bubbles exist in the column it is necessary to remove these before use.

a. Remove a column from the package.
b. Remove the top cap only from the column. Leave the bottom cap in place.
c. Cut the cap off of a Collection Tube and insert the column in the tube. Place in a microcentrifuge.
d. Centrifuge at 8,000 rpm (~5,500 x *g*) for 5 minutes.
e. Examine the column to verify homogeneity of the packed trypsin-silica resin. Repeat the centrifugation if necessary to remove any remaining bubbles.
   Note: Save the Collection Tube for the column wash and equilibration.

3. Column Equilibration

The glycerol must then be removed and the column equilibrated in Enzyme Reaction Buffer. Washing with water after equilibration lowers the salt concentration in the final digest solution.

a. Remove the bottom cap from the column.
b. Place the column in the Collection Tube used during bubble removal from the column.
c. Centrifuge at 3,000 rpm (~800 x *g*) for 2-3 minutes.
d. Apply 200 µl of Column Wash Buffer to the top of the resin bed.
e. Centrifuge at 3,000 rpm (~800 x *g*) for 2-3 minutes.
f. Repeat the 200 µl wash (steps d and e) once using Enzyme Reaction Buffer alone (No denaturant).
g. Apply 200 µl of ultrapure water to the top of the resin bed.
h. Centrifuge at 3,000 rpm (~800 x *g*) for 2-3 minutes.
i. Repeat the 200 µl water wash (steps g and h) one to two times.
j. Centrifuge for a final 5 minutes at 8,000 rpm (~5,500 x *g*) to remove residual liquid from the column.
k. Discard the washes.

The column should be used the same day it is prepared.

Sample Digestion

The resin volume in the column is approximately 150 µl.

1. Dissolve or dilute the protein to be digested in denaturant to attain a final protein concentration of approximately 4 mg/ml. Protein Extraction Reagent Type 4 (Product Code C0356) is recommended as the denaturant. To fully denature the proteins, mix gently and incubate at room temperature for 10 minutes.
2. If desired, reduce and alkylate the protein. The use of the ProteoPrep Reduction and Alkylation Kit (Product Code PROTRA) is recommended.
3. Dilute the protein solution with 3 volumes of Enzyme Reaction Buffer. If a lower salt concentration is desired, add enough Enzyme Reaction Buffer to adjust the pH of the protein solution to ~8 and complete the dilution with water.

4. Apply up to 100 µl (100 µg of protein) of the diluted protein solution to the top of the prepared spin column. Note that as little as 4 µg of protein may be applied to the column.
5. Place the spin column in a clean collection tube.
6. To move the protein solution into the resin bed centrifuge at 1,000 rpm (~100 x *g*) for 30 seconds. Note: If a variable speed centrifuge is not available, the protein solution can be moved into the resin bed by applying direct pressure with a pipette.
7. Examine the column to verify that there is no liquid remaining on top of the column and that <25 µl has passed through the column into the collection tube.
   - If residual liquid is present on top of the resin, centrifuge at 1,000 rpm (~100 x *g*) for another 30 seconds or as needed until all liquid is fully in contact with the resin.
   - If it appears that >50% of the liquid applied to the column has passed through the resin, it may be advantageous to reapply the flow-through.
8. Incubate at room temperature for a total of 15 minutes from the start of application of the protein sample to the column. For most proteins tested, the digestion is essentially complete within 15 minutes of contact time with the resin. Proteins that are difficult to denature may require longer digestion times up to 30 minutes. Some proteins can be digested in 5 minutes or less.
9. Elute the peptides by applying 150 µl of Enzyme Reaction Buffer or water to the top of the column and centrifuging at 3,000 rpm (~800 x *g*) for 2 minutes. Using water rather than Enzyme Reaction Buffer will result in a sample containing less salt. This procedure will remove ~90% of the peptides from the column. The recovered digest may be reapplied to the column if further proteolysis is required.

The peptides are now ready for mass spectrometric analysis. The sample may be acidified, desalted, or concentrated prior to analysis, as desired.

Some proteins are inherently resistant to proteolysis by trypsin and are better digested by other enzymes. The Protease Profiler™ Kit (Product Code PP0500) contains Asp-N, Glu-C, Lys-C and Arg-C as well as trypsin, and may be used for further evaluation.

Multiple Uses of Trypsin Spin Columns

The column can be used more than once with aliquots from the same sample source. To employ this option after the initial digestion, wash the column once with 200 µl of Enzyme Reaction Buffer to ensure the column is at the optimal pH, then wash twice with 200 µl of water to remove excess buffer. Centrifuge at 5,000 rpm (~2,300 x *g*) for 5 minutes to remove residual liquid from the column. Discard the washes. Prepare the protein sample as before (Sample Digestion, steps 1-3) and apply up to 100 µl to the column and repeat the digestion. The columns may be used at least 4 times with no loss in performance.

References

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5. Stone, K.L., *et al.*, A Practical Guide to Protein and Peptide Purification for Microsequencing, Academic Press, Inc. (New York, NY: 1989) p. 31.

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