

Pierce™ Peptide Desalting Spin Columns

Catalog Numbers 89851 and 89852

Doc. Part No. 2162704 Pub. No. MAN0017280 Rev. A.0

Contents

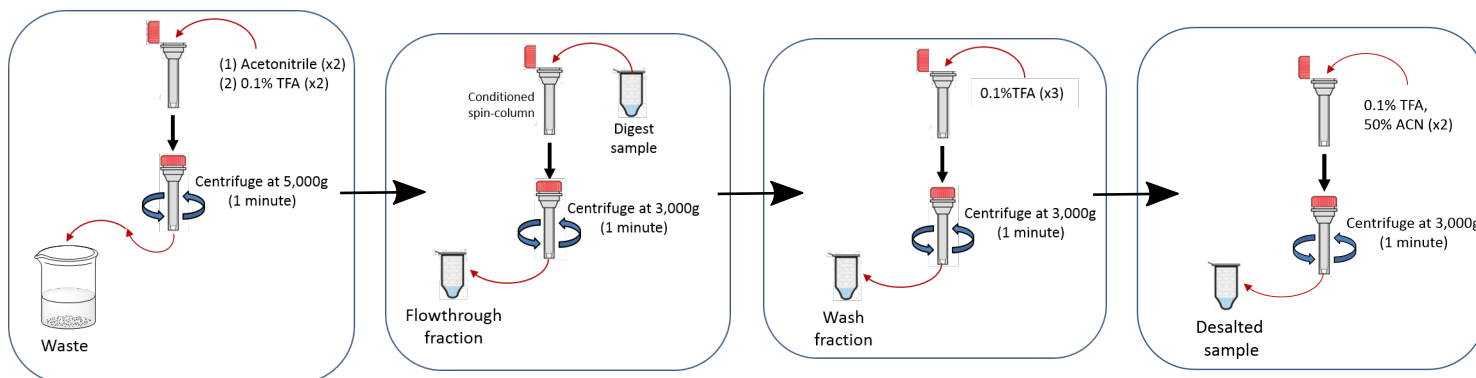
Product	Cat. No.	Contents	Storage
Pierce™ Peptide Desalting Spin Columns	89852	25 spin columns containing 20 mg of resin in a 1:1 water/DMSO slurry	Store at 2-8°C.
	89851	50 spin columns containing 20 mg of resin in a 1:1 water/DMSO slurry	

Product description

The Thermo Scientific™ Pierce™ Peptide Desalting Spin Columns are optimized for desalting 5 µg to 5 mg of peptide sample from protein digests. The polymer-based hydrophobic resin contained in the column is stable from pH 1-14 and binds peptide samples with excellent recovery (typical recovery is >90%) under reversed phase conditions. Columns can also be used for removal of excess hydrolyzed/quenched TMT reagents after labeling to enable efficient and reliable downstream LC-MS analysis.

Procedure summary

Proteolytic digests of proteins extracted from cells or tissues are loaded onto an equilibrated desalting spin column. Peptides are bound to the hydrophobic resin under acidic aqueous conditions and desalted by washing the column with acidified water by low-speed centrifugation. A solution with acidified organic solvent (0.1% TFA, 50% acetonitrile) is then applied to the column to elute bound peptides by centrifugation.



Additional materials required

- Trifluoroacetic acid (TFA) (Product No. 28904)
- Acetonitrile (ACN), LC-MS Grade (Product No. 51101)
- Water, LC-MS Grade (Product No. 51140)
- Methanol (MeOH), Optima™ LC-MS Grade (Fisher Scientific, Product No. A456-1)
- Thermo Scientific™ Pierce™ Low Protein Binding Microcentrifuge Tubes, 2.0 mL (Product No. 88379 or 88380)
- Microcentrifuge with adjustable rotor speed up to 7,000 × g
- Vacuum centrifuge

Additional information

- Do not exceed the recommended centrifugation speeds as this may damage the column frit. Damaged frits may result in resin leakage, leading to sample loss and/or damage to the LC system.
- Use low protein-binding microcentrifuge tubes to ensure maximum sample recovery.
- Avoid sample contamination and direct skin contact with solvents and chemicals. Always wear gloves when handling the spin columns and samples.

Prepare materials

1. **Conditioning/Washing Solution (0.1% TFA in water)** — Each sample requires 1.5 mL for processing. Add 2 μ L of trifluoroacetic acid to 2 mL water.
2. **Elution Solution (0.1% TFA, 50% ACN)** — Each sample requires 600 μ L for elution. Add 2 μ L of trifluoroacetic acid to 1 mL water and 1 mL acetonitrile. Mix well.
3. *(Optional)* **TMT Washing Solution (5% MeOH, 0.1% TFA in water)** — Each sample will require 300 μ L of this solution for processing. Add 50 μ L of methanol and 1 μ L of trifluoroacetic acid to 950 μ L of water and mix well.

Clean up samples

Condition the spin columns

1. Remove the white tip from the bottom of the column and discard. Place the column into a 2 mL microcentrifuge tube.
2. Centrifuge at 5,000 \times g for 1 minute to remove the solution and pack the resin material. Discard the liquid.
Note: Do not exceed recommended centrifugation speeds.
3. Remove the top screw cap and load 300 μ L of ACN into the column. Replace the cap, place the spin column back into a 2 mL microcentrifuge tube and centrifuge at 5,000 \times g for 1 minute. Discard ACN and repeat the wash step.
4. Wash the spin column twice with 0.1% TFA in water, as described in step 3. The column is now conditioned and ready for use.

Bind and wash samples

1. Dissolve 5-5,000 μ g of digested sample in 300 μ L of 0.1% TFA in water.
Note: Peptide samples need to be completely dissolved and free of organic solvent (e.g., ACN, DMSO, etc.).
2. Place the spin column into a 2 mL microcentrifuge tube. Load 300 μ L of the sample solution onto the column, replace the top cap and centrifuge at 3,000 \times g for 1 minute. Discard the flowthrough.
3. Place the column into a 2 mL microcentrifuge tube. Load 300 μ L of 0.1% TFA solution onto the column and centrifuge at 3,000 \times g for 1 minute to collect the wash. Discard the eluate and repeat this step 2 more times for a total of 3 washes.
Note: TMT-labeled samples require 2 additional column washes with 300 μ L of 5% MeOH, 0.1% TFA solution to completely remove the excess TMT reagent.

Elute desalted peptides

1. Place the column into a new 2 mL microcentrifuge tube. Load 300 μ L of the 50% ACN, 0.1% TFA solution and centrifuge at 3,000 \times g for 1 minute to collect the eluate.
2. Apply another 300 μ L of the 50% ACN, 0.1% TFA solution and centrifuge at 3,000 \times g for 1 minute, collecting the eluate into the same microcentrifuge tube.
3. Evaporate the liquid contents of each sample tube to dryness using vacuum centrifugation (e.g., SpeedVac concentrator).
4. Re-suspend dry samples in an appropriate volume of 0.1% formic acid (FA) before LC-MS analysis.
5. *(Optional)* Determine the peptide concentration and yield with a peptide quantitation assay.

Related products

Product	Cat. no.
BCA Protein Assay Kit	23227
Pierce™ Quantitative Fluorometric Peptide Assay	23290
Pierce™ Quantitative Colorimetric Peptide Assay	23275
TMTsixplex™ Isobaric Label Reagent Set	90061
TMTsixplex™ Isobaric Mass Tagging Kit	90064
TMT10plex™ Isobaric Label Reagent Set	90110
TMT10plex™ Isobaric Mass Tagging Kit	90113
iodoTMTsixplex™ Label Reagent Set	90101
iodoTMTsixplex™ Isobaric Mass Tag Labeling Kit	90103
aminoxyTMTsixplex™ Label Reagent Set	90401
Pierce™ Mass Spec Sample Prep Kit for Cultured Cells	84840
Pierce™ HeLa Digest Protein Standard	88328
Pierce™ Trypsin Protease, MS Grade	90057
Trifluoroacetic Acid, Sequencing Grade	28904
Water, LC-MS Grade	51140
Acetonitrile (ACN), LC-MS Grade	51101

Troubleshooting

Observation	Possible cause	Recommended action
Low peptide yields.	Low protein yield following lysis and the protein extraction procedure.	Estimate protein concentration using Thermo Scientific™ BCA Protein Assay Kit (Product No. 23227). Use an alternative protein extraction procedure.
	Lyophilized/dried peptide samples were not completely solubilized before sample loading onto the spin column.	Increase vortexing/sonication time to completely dissolve the dried peptide sample.
	Organic solvents were not completely removed before loading sample onto the column.	Ensure the sample is completely dry before resuspending in 0.1% TFA solution.
	Samples were not properly acidified prior to loading onto the column.	Check pH of the sample solution. For the best retention of peptides, pH should be between 2.5-3.5. Adjust pH if necessary with a dilute formic acid, acetic acid, or trifluoroacetic acid solution.
Unsuccessful recovery.	Incorrect centrifuge speeds were used.	Ensure a proper centrifuge speed is used. To convert from revolutions per minute (rpm) to <i>g</i> , use the following formula: $g = (1.118 \times 10^{-5}) RS^2$ where <i>g</i> is the relative centrifugal force, R is the rotor radius in centimeters, and S is the centrifuge speed in rpm. For example, centrifugation of a sample at 5,000 rpm in a microcentrifuge having a rotor radius of 7 cm will deliver a centrifugal force of $1,957 \times g$. Note: Calculated centrifuge speed is only written in the form of (XXXX × <i>g</i>).
	Dried, cleaned peptide samples are not completely recovered from the sample tube.	Increase vortexing/sonication time to completely dissolve the dried peptide sample. For smaller sample loads, repeated aspiration of dissolving solution using a pipette may improve recovery.
Low peptide/protein identification numbers.	Sample load was too low (<5 µg).	Estimate peptide concentration using the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay (Product No. 23290) or Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay Kit (Product No. 23275).
		Use low protein-binding tubes for handling of the samples and fraction collection.
	Incorrect chromatography or mass spectrometer instrument settings were used.	Consult instrument user manuals or online resources to determine the optimal instrument settings for your system. Verify LC-MS system performance with the Thermo Scientific™ Pierce™ HeLa Digest Protein Standard (Product No. 88328).



Manufacturer: Pierce Biotechnology, Inc. | Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

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