

Store at
4°C and RT

#41745

Peptide Desalting C18 StageTip and Buffer Kit

1 Kit
(10 assays)



Cell Signaling

TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Kit Quantity	Storage Temp
Water, LC-MS Grade (Burdick and Jackson)	27732	2 x 1.5 mL	RT
Acetonitrile	40644	1 x 1 mL	4°C
1% TFA	50573	1 x 1 mL	4°C
Peptide Desalting C18 StageTip	45943	10	RT

Description: Peptide Desalting C18 StageTips are solid phase extraction tips that bind to peptides. This kit provides 10 individual StageTips with the necessary solutions to concentrate and purify peptides eluted from PTMScan® beads prior to liquid chromatography-mass spectrometry (LCMS) analysis.

Background: Reversed-phase chromatography is an important technique for analytical and preparative biochemical separation and purification. Hydrophobic peptides can be concentrated and purified by reversed-phase chromatography with excellent recovery. Peptide Desalting C18 StageTips use the same basic mechanism of reversed-phase chromatography in a convenient format to process peptide samples for LCMS analysis (1).

Storage: All components in this kit are stable for at least 12 months when stored at the recommended temperature.

Please visit cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

(1) Rappsilber, J. et al. (2003) *Anal Chem* 75, 663-70.

Base Peak Chromatograms



MS1 Peak Area of ~ 1,000 Peptides



Base peak chromatograms (left) of tryptic peptides derived from human MKN-45 gastric carcinoma cancer cell line that were prepared with Peptide Desalting C18 StageTips. Approximately 110 ng of peptides were analyzed on Orbitrap Q Exactive mass spectrometer and resolved using a 45 min reversed-phase gradient from 7.5% to 32% acetonitrile on a C18 column. MS1 peak areas (right) of approximately 1,000 peptides randomly selected from the StageTip desalted dataset were compared using Skyline software between two replicates.

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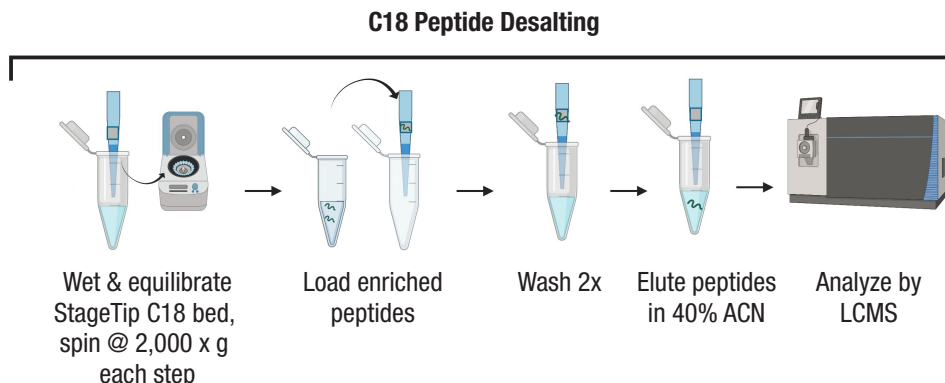
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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry FC-FP—Flow cytometry-Fixed/Permeabilized FC-L—Flow cytometry-Live E-P—ELISA-Peptide
Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse
All—all species expected. Species enclosed in parentheses are predicted to react based on 100% homology.

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General Peptide Desalting Protocol with Buffers



Concentration and Purification of Peptides for LCMS Analysis on C18 Tip

NOTE: There are many routine methods for concentrating peptides using commercial products, such as C18 tips, that have been optimized for peptide desalting/concentration. Regardless of the particular method, we recommend that the method of choice be optimized for recovery and be amenable to peptide loading capacities of at least 10 µg input sample, for instance eluate from PTMScan® immunoaffinity purification (IAP).

A. Solutions and Reagents

NOTE: Prepare all solutions in containers that have never been autoclaved or exposed to soap, as detergents will interfere with LCMS analysis. All percentage specifications for solutions below are volume/volume.

1. StageTip Wetting Solution (50% Acetonitrile, 0.1% TFA) = 25 µL of Acetonitrile #40644, 20 µL of LC-MS Grade Water #27732, and 5 µL of 1% TFA #50573 for 50 µL final volume per assay.
2. StageTip Equilibration and Washing Solution (0.1% TFA) = 180 µL of LC-MS Grade Water #27732 and 20 µL of 1% TFA #50573 for 200 µL final volume per assay.
3. StageTip Elution Solution (40% Acetonitrile, 0.1% TFA) = 8 µL of Acetonitrile #40644, 10 µL of LC-MS Grade Water #27732, and 2 µL of 1% TFA #50573 for 20 µL final volume per assay.

NOTE: Organic solvents are volatile. Tubes containing small volumes of these solutions should be prepared immediately before use and should remain capped as much as possible to prevent evaporation of organic components.

Equipment Not Included:

- 1.5 mL microcentrifuge tubes
- Centrifuge capable of handling 1.5 mL tubes
- Peptide Desalting C18 StageTip Adaptors #58992
- Vacuum concentrator (Speed-Vac)

B. Procedure

NOTE: All centrifugation steps in this section should be carried out at room temperature. Spin at a speed up to 2,000 x g, such that all of the solution passes through the tip in approximately 3 min. Confirm that all of the solution has passed through after each step, and if necessary, spin for a longer duration until the entire solution passes through the tip

1. Cut the lid off of a 1.5 mL tube and place an adaptor on top. Place a C18 tip in the adaptor #58992. There should be enough room in the tube to collect ~100 µL of liquid without touching the C18 material at the bottom of the tip.
2. Equilibrate the C18 tip by pipetting 50 µL of StageTip Wetting Solution into the tip and centrifuging. Transfer the C18 tip to a clean collection tube.
3. Add 50 µL of StageTip Equilibration and Washing Solution and centrifuge. Repeat this step once for two total Equilibration and Washing steps. Discard flow-through.
4. Load sample by centrifuging IAP eluate (approximately 100 µL initial total volume) through the C18 tip. Load IAP eluate 50 µL at a time for a total of two loading steps. Users may reload the same 50 µL of eluate that passed through the C18 tip one additional time for a total of four loading steps. Peptides will be bound to the C18 tip. Discard flow-through after loading is complete.
5. Wash the C18 tip by passing 50 µL of StageTip Equilibration and Washing Solution. Repeat this step once for two total Equilibration and Washing steps. Discard flow-through and transfer the C18 tip to a clean collection tube.
6. Elute peptides off the C18 tip by centrifuging 10 µL of StageTip Elution Solution. Repeat this step once for two total elutions. Confirm that all of the solution has passed through after each elution step. Pool the two eluates for a final volume of 20 µL.
7. Dry down the C18 tip eluate from the C18 tip purification in a vacuum concentrator (Speed-Vac) and store the dry peptides at -20°C until LCMS analysis will begin. At that time, re-dissolve the peptides in an appropriate solvent for LCMS analysis, such as 5% acetonitrile, 0.1% TFA, 94.9% water (MS grade).