Store at RT

# Peptide Desalting C18 StageTip

#45943

Small 96 assays
Petite 10 assays

**Support:** +1-978-867-2388 (U.S.)
cellsignal.com/support

**Orders:** 877-616-2355 (U.S.)
orders@cellsignal.com

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

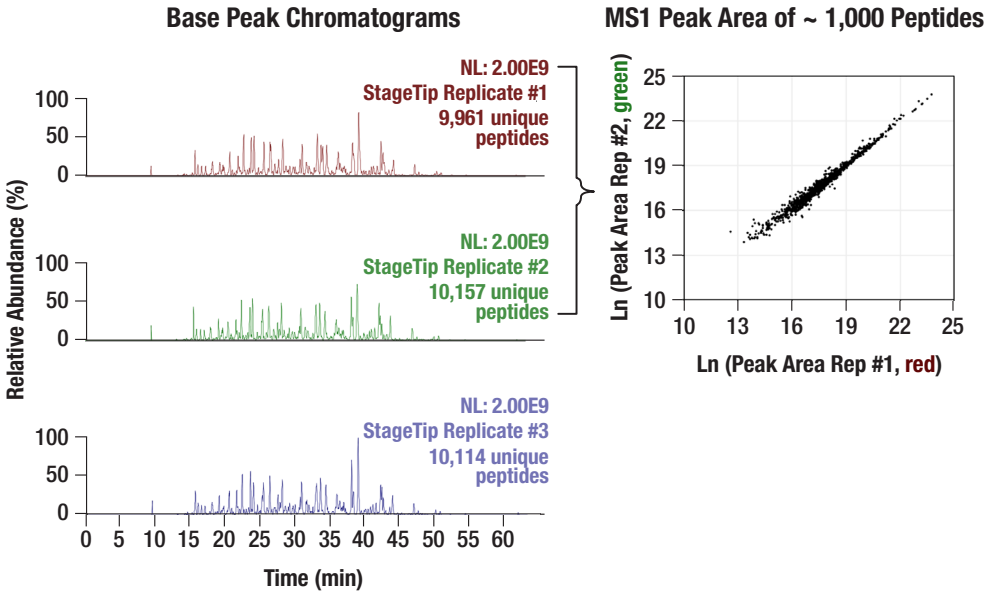
**Description:** Peptide Desalting C18 StageTips are solid phase extraction tips that bind to peptides. This product is available in two sizes 10 or 96 individual StageTips that can be used to concentrate and purify peptides eluted from PTMScan® beads prior to liquid chromatography-mass spectrometry (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:** This product is stable for at least 36 months when stored at the recommended temperature. Store at room temperature.

**Background Reference:**

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




*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 unique peptides randomly selected from the StageTip desalted dataset were compared using Skyline software between two replicates.*



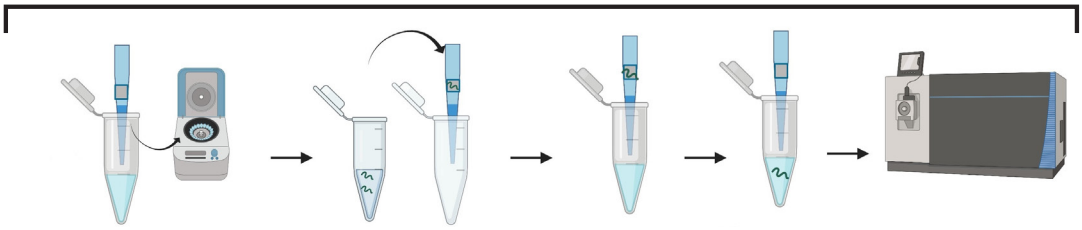




**Applications:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
**Species Cross-Reactivity:** **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.

# General Peptide Desalting Protocol

## C18 Peptide Desalting




### 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 solutions with LCMS grade water (Cell Signaling Technology, #27732). Organic solvents (trifluoroacetic acid, acetonitrile) should be of the highest grade.

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 are volume/volume.

1. StageTip Wetting Solution
   (0.1% trifluoroacetic acid, 50% acetonitrile)
2. StageTip Equilibration & Washing Solution
   (0.1% trifluoroacetic acid)
3. StageTip Elution Solution
   (0.1% trifluoroacetic acid, 40% acetonitrile)

   **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
- StageTip adaptors (Cell Signaling Technology, #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. If necessary, spin for longer duration until entire solution passes through 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 (Cell Signaling Technology, #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 at 2,000 x g for approximately 3 min or until all of the solution has passed through. Transfer the C18 tip to a clean collection tube.
3. Add 50 µL of StageTip Equilibration & Washing Solution and centrifuge 3 min or until all of the solution has passed through. Repeat this step once for two total Equilibration & 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 re-load 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. Confirm all of the solution has passed through after each loading step.
5. Wash the C18 tip by passing 50 µL of StageTip Equilibration & Washing Solution. Repeat this step once for two total Equilibration & Washing steps. Confirm all of the solution has passed through after each wash step. 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 all of the solution has passed through after each elution step. Pool the two resulting 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 dried peptides at -20°C until LCMS analysis begins. 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).