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PTMScan® Peptide Purification Kit

Cell Signaling
TECHNOLOGY®

#35741

1 Kit
(10 Purifications)Support: +1-978-867-2388 (U.S.)
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New 11/18

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Kit Quantity
PTMScan® C18 Reversed-Phase Columns	73647	10 each
PTMScan® Sample Reservoir Syringes	49639	10 each

Description: C18 Reversed-Phase Columns are useful tools for purifying and desalting peptides in preparation for PTMScan® protocols and other mass spectrometry applications.

Background: Reversed-phase chromatography is an important technique for analytical and preparative biochemical separation and purification. Hydrophobic proteins, peptides, and nucleic acids can be separated by reversed-phase chromatography with excellent resolution and recovery. Furthermore, the use of ion pairing modifiers in the mobile phase allows reversed-phase chromatography of charged solutes such as hydrophilic peptides and fully deprotected oligonucleotides. Preparative reversed-phase chromatography has been used in applications ranging from micro-purification of protein fragments for sequencing (1) to process scale purification of recombinant protein products (2) and mass spectrometry proteomic analysis (3).

Notes:

- Approximately 20 mg of protease digested peptides can be purified on one C18 reversed-phase column.
- The steps described in the Directions for Use section use gravity feed. In cases where the flow rate has dramatically decreased, you may apply gentle pressure to the column using the syringe plunger.
- Each time a solution is applied to the column, an air bubble can form at the narrow junction between the syringe and the column inlet. This will decrease the flow rate considerably. The bubble should be removed by flushing with a gel loading tip fitted on a P200 pipette.

Directions for Use:

1. Acidify the peptides prior to loading by adding 1/20 volume of 20% TFA for a final concentration of 1% TFA.
2. Clarify the peptide sample by centrifuging at 1,780 x g at room temperature for 15 minutes.
3. Remove and save the plunger from a 10 ml syringe and connect to the short end of the C18 column.
4. Pre-wet the C18 reversed-phase column with 5 ml of 100% acetonitrile.
5. Equilibrate the column sequentially with three washes of 0.1% TFA using 1, 5, and 6 ml.
6. Load the acidified peptide sample onto the column; the peptides will bind to the C18 material.
7. Wash the column sequentially with three washes of 0.1% TFA using 1, 5, and 6 ml.
8. Wash the column with 2 ml of 5% acetonitrile with 0.1% TFA.
9. Place each column above a new, clean 50 mL collection tube.
10. Elute the peptides 3 times with 3 ml of 40% acetonitrile with 0.1% TFA.
11. Eluted peptides should then be frozen completely to proceed to the lyophilization step. Freeze for 4-16 hours in -80°C freezer or on a dry ice and ethanol bath.

See specific PTMScan® protocol for further details.

Storage: Columns and syringes can be stored in the original sealed pouch for up to 24 months at room temperature. For best results, open pouches should be closed and sealed with tape, store resealed package in a desiccator for up to 6 months.

Background References:

- (1) Renlund, S. et al. *Peptides* 14, 1125-32.
- (2) Olson, C.V. et al. (1994) *J Chromatogr A* 675, 101-12.
- (3) Rush, J. et al. (2005) *Nat Biotechnol* 23, 94-101.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry 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.