Store at RT and 4°C

#85106

PTMScan® HS Immunoenrichment Companion Reagents Kit

1 Kit
(10 assays)

Support: +1-978-867-2388 (U.S.)
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New 12/20

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

Components Ship As: 87029S	Item #	Kit Quantity	Storage Temp
S-Trap™ Midi Spin Columns	88371	1 x 10 Pack	RT
S-Trap™ SDS Solubilization Buffer	44386	1 x 5 mL	RT
S-Trap™ Phosphoric Acid	63866	1 x 1 mL	RT
S-Trap™ Binding/Wash Buffer	25904	6 x 25 mL	RT
S-Trap™ Elution Buffer #1 (TEAB)	35234	1 x 8.5 mL	RT
S-Trap™ Elution Buffer #2 (TFA)	64820	1 x 5 mL	RT
S-Trap™ Elution Buffer #3 (ACN/TFA)	84467	1 x 5 mL	RT
Water, LC-MS Grade (Burdick and Jackson™)	27732	1 x 21 mL	RT
Components Ship As: 73111S	**Item #**	**Kit Quantity**	**Storage Temp**
PTMScan® Trypsin, TPCK-Treated	56296	1 x 1 mg	4°C
Phosphatase Inhibitor Cocktail (100X)	5870	1 x 200 µl	4°C
Iodoacetamide, PTMScan® Qualified	88931	5 x 3.7 mg	4°C
DTT (Dithiothreitol)	7016	1 x 192.8 mg	4°C

Description: The PTMScan® HS Immunoenrichment Companion Reagents Kit includes ten S-Trap™ Midi Spin Columns and all the necessary reagents to prepare purified peptides ready for posttranslational modification enrichment. Cells or tissues from any species are supported. The kit includes supplies to prepare ten trypsin digestions of 1 mg each, which is sufficient for ten immunoenrichments with a PTMScan® HS Antibody Kit. The soluble protein content of cells and tissues vary, but 1 x 10^7 cells or 15-30 mg of wet tissue from mammalian sources should be sufficient. Clean peptides are ready for PTM enrichment with only one day of hands-on work and one day of hands-off drying time.

Background: Proteomics analyses of posttranslationally modified peptides rely on both high-quality enrichments and pre-enrichment sample preparation. Suspension Traps (S-Trap™ columns) are a convenient tool to combine protein digestion and peptide cleanup into a single procedure. The S-Trap™ column is a spin filter made out of quartz that can trap large particles, such as precipitated proteins, but has little to no affinity for smaller particles, such as peptides (1). Cells and tissues can be prepared in any lysis buffer of choice, but buffers containing SDS are reported to extract more protein per cell than other buffers and help to maximize the performance of S-Trap™ columns (2). Acidifying the cell lysate and mixing it with methanol causes the proteins to precipitate, so that they can be captured on the S-Trap™ column with a brief centrifugation step. Detergents, along with lipids and any other contaminants, are washed away with additional methanol. The captured proteins can then be digested with trypsin or any other appropriate enzyme. Digesting on a solid substrate such as the S-Trap™ column enhances the enzymatic efficiency, reducing missed cleavages in comparison to in-solution methods (3). The peptides elute easily into mass spectrometry-friendly solutions. Once dried, these purified peptides are ready to be enriched for any PTM of interest using techniques such as IMAC #20432 or PTMScan®. If desired, the peptides are also ready for direct mass spectrometry analysis without further cleanup.

The S-Trap™ workflow is much faster and less laborious than in-solution digestion because it avoids a separate peptide purification procedure. Once protein extracts are collected, reduction and alkylation takes 45 minutes, loading and washing the S-Trap™ columns takes 15 minutes, and then the samples are allowed to digest overnight. Clean peptides are collected the next morning in less than 15 minutes. Drying in a vacuum concentrator (or lyophilizer) can be completed over the next night, so that enrichment can be completed on the third day of sample preparation.

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

Background References:

(1) Zougman, A. et al. (2014) *Proteomics* 14, 1006-0.

(2) Elinger, D. et al. (2019) *J Proteome Res* 18, 1441-5.

(3) Ludwig, K.R. et al. (2018) *J Proteome Res* 17, 2480-90.

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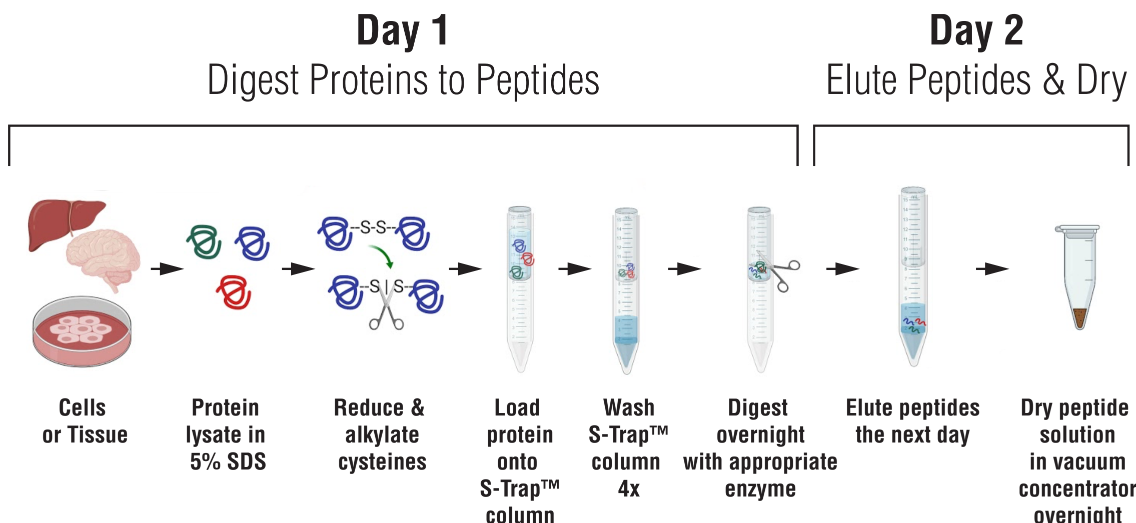
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.

S-Trap™ Sample Preparation Protocol



Reagents not supplied and equipment required:

1. Phosphate Buffered Saline (PBS-20X) (#9808)
2. Protein Quantitation Colorimetric Assay Kit, such as BCA Protein Assay Kit (#7780)
3. 1mM Hydrochloric acid (HCl)
4. Centrifuges capable of handling 15mL tubes and 1.5mL tubes
5. Vacuum concentrator

A Solutions and Reagents

NOTE: The S-Trap™ Midi Spin Columns can support the digestion of 0.3 – 5 mg soluble protein. Approximately 1 mg is recommended for a PTMScan® HS experiment.

1. **1X S-Trap™ SDS Solubilization Buffer:** Mix 5 mL of S-Trap™ SDS Solubilization Buffer (2X) with 5 mL of DI water to make Solubilization Buffer 1X. Store at room temperature; the SDS will precipitate if stored cold. Prior to each experiment, for each sample, take out 1 ml of 1X S-Trap™ SDS Solubilization Buffer and mix with 20 uL of Phosphatase Inhibitor Cocktail (100X), which should be used at 1/50 for PTMScan® experiments.
2. **Dithiothreitol (DTT):** Make 1.25 M stock: Resuspend one tube containing 192.8 mg with 1 mL DI water. Divide into 25 uL aliquots. Store at -20°C for up to three months. Thaw one aliquot for each experiment.
3. **Iodoacetamide solution:** Dissolve one vial in 200 uL of DI water immediately before use. Each vial is sufficient to alkylate four samples or 2mL of lysate. Keep the solution protected from light.
4. **Trypsin stock solution:** Dissolve the vial of trypsin powder in 1mL of 1mM HCl. to make a 1 mg/mL stock. Divide into 100 uL aliquots and store at -80 °C for up to one year.

B Preparation of Cell Lysate, Cultured Cells

1. Grow approximately 1-4 x 10^7 cells for each experimental condition (enough cells to produce approximately 1-2 mg of soluble protein). The cell number corresponds to approximately one 150 mm culture dish (depending on the cell type).
2. Wash adherent cells in cold PBS, then harvest by scraping in 500 uL of 1X S-Trap™ SDS Solubilization Buffer per plate. For suspension cells, collect the cells by centrifugation and wash the pellet with cold 1X PBS. Harvest the pellet with 500 uL of 1X S-Trap™ SDS Solubilization Buffer. Do not cool lysate on ice as this may cause precipitation of the SDS. The harvested cells can be frozen and stored at -80°C for several weeks or proceed to sonication. (**SAFE STOP**)
3. Using a sonicator with a microtip, sonicate lysate at 5 W output with 3 bursts of 15 sec each. Cool on ice for 1 min between each burst. Clear the lysate by centrifugation at 10,000 x g for 15 min at room temperature and transfer the protein extract (supernatant) into a new tube. Store the cleared lysate at -80°C at this point or continue directly through the Protein Quant and Digestion steps. (**SAFE STOP**)

   NOTE: Lysate sonication fragments DNA and reduces sample viscosity. Ensure that the sonicator tip is submerged in the lysate. If the tip is not submerged properly, it may induce foaming and degradation of your sample.

   NOTE: Centrifugation is performed at room temperature to prevent SDS from precipitating out of solution.

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C Preparation of Tissue Lysates

1. Select a fresh-frozen piece of tissue and place it in a 50 mL conical tube. Add approximately 2 mL of 1X S-Trap™ SDS Solubilization Buffer per 100 mg of wet tissue, or enough to submerge it completely.
2. Lyse the tissue using a homogenizer. Wash the tool with DI water in between each sample. Keep the moving parts submerged in buffer to avoid excessive foaming.
3. Using a sonicator with a microtip, sonicate lysate at 5 W output with 3 bursts of 15 sec each. Cool on ice for 1 min between each burst. Clear the lysate by centrifugation at 10,000 x g for 15 min at room temperature and transfer the protein extract (supernatant) into a new tube. Store the cleared lysate at -80°C at this point or continue directly through the Protein Quant and Digestion steps. (**SAFE STOP**)

   NOTE: Lysate sonication fragments DNA and reduces sample viscosity. Ensure that the sonicator tip is submerged in the lysate. If the tip is not submerged properly, it may induce foaming and degradation of your sample.

   NOTE: Centrifugation is performed at room temperature to prevent SDS from precipitating out of solution.

D. Protein Quantitation

1. Measure soluble protein concentration using a colorimetric assay kit that is compatible with the SDS lysis buffer. The bicinchoninic acid (BCA) assay is recommended.
2. Normalize all samples so that equal amounts of protein are prepared for each condition and replicate. Alternatively, design the experiment so that equal numbers of cells are prepared for each sample.

E. Reduction and Alkylation of Proteins

1. Add 1/278 volume of 1.25 M DTT to the cleared cell supernatant to reach 4.5 mM DTT final concentration (e.g. 1.8 µL of 1.25 M DTT for 500 uL of protein extract). Mix well, and place the tube into a 55°C incubator for 30 min.
2. Cool the solution on ice briefly until it has reached room temperature.
3. Add 1/10 sample volume of iodoacetamide solution to the cleared cell supernatant, mix well, and incubate for 15 min at room temperature in the dark.

F. S-Trap™ Column Cleanup and Protease Digestion

1. Add 1/10 sample volume of S-Trap™ Phosphoric Acid to the sample and mix well.
2. Transfer the sample to a 15 mL tube and add 6.6x the sample volume (eg. 3.3 mL) of S-Trap™ Binding/Wash Buffer. Mix well. A cloudy protein colloid should form immediately.

   IMPORTANT: The protein in the sample is insoluble in the S-Trap™ Binding/Wash Buffer . DO NOT CENTRIFUGE the sample or allow it to settle before loading onto the S-trap™ column in the next step.
3. Load the full volume of sample onto the S-Trap™ column, ensuring all the cloudy precipitate is transferred. Centrifuge 2 min at 4000 x g at room temperature until all the solution has passed through. Discard the flow-through.

   NOTE: All centrifugation steps for S-Trap™ columns should be performed at room temperature and 4000 x g.
4. Wash the S-Trap™ column with 3 mL of S-Trap™ Binding/Wash Buffer and centrifuge 2 min. Discard the flow-through. Repeat 3 times for 4 washes total.
5. Transfer S-Trap™ columns to clean collection tubes prior to digestion.
6. Prepare 350 uL of trypsin working solution for each sample. Use trypsin at 1:10 (w/w) enzyme : substrate (eg. 100 ug trypsin for 1 mg sample). Dilute 1 mg/mL trypsin stock up to 350 uL total with S-Trap™ Elution Buffer #1 (TEAB) and verify that the pH is ~8. The volume of digestion solution can be increased to ~500 uL if more trypsin stock is needed.
7. Add the 350 uL trypsin solution to each cartridge and cap the tubes LOOSELY so that the digestion solution can absorb into the cartridge without creating negative air pressure. The cartridges may be centrifuged briefly but transfer any liquid that passes through back to the top of the cartridge. Place them in an incubator or water bath overnight at 37°C.

   IMPORTANT: Do not shake the tubes. Set the tubes in a rack so they remain secure and straight upright and the enzyme solution will absorb evenly into the cartridge bed.

   IMPORTANT: Complete tryptic digestion is critical for maximum peptide recovery in the following elution steps. Cutting incubation time short or using less than the recommended amount of trypsin will reduce performance.
8. The next day, add 500 uL of S-Trap™ Elution Buffer #1 (TEAB) to the S-Trap™ column and centrifuge 1 min or until all solution passes through at 4000 x g. Keep the cartridge in the same collection tube for all three elution steps (see steps 9 & 10).
9. Add 500 uL of S-Trap™ Elution Buffer #2 (TFA) to the S-Trap™ columns, centrifuge 1 min or until all the solution passes through, at 4000 x g.
10. Add 500 uL of S-Trap™ Elution Buffer #3 (ACN/TFA) to the S-Trap™ columns, centrifuge 1 min.
11. Verify that the combined elutions have a pH<3. Transfer the eluate (1.85 mL altogether) to a 2 mL microcentrifuge tube.

    NOTE: At this stage, the peptides are clean for LCMS if analysis of the unmodified proteome is desired. 1% of the total volume can be set aside for this purpose.
12. Dry the peptide solution in a vacuum concentrator set to ambient temperature overnight or until completely dry. The pellet should be visible at the end.

    NOTE: Peptide solutions may be frozen at -80°C for 1 hr or longer before placing in the vacuum concentrator; this will prevent full tubes from spilling when placed at an angle to dry. (**SAFE STOP**)

    NOTE: A standard lyophilization apparatus is also acceptable in place of a vacuum concentrator.

    NOTE: Dry, digested peptides are stable at -80°C for several months (seal the closed tube with parafilm for storage). (**SAFE STOP**)

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