MANUAL

Biotin S-Cap (SUMO Capture Reagent)

Catalog Number: SM-101

Background:

Small ubiquitin-like modifier (SUMO) proteins are a family of proteins that have a similar structure with ubiquitin. There are four SUMO genes in human genome. SUMO2 and SUMO3 are highly similar (97% identical), while SUMO1 is quite distinct from other members. SUMO4 contains a specific proline 90 residue, which prevents it from being processed by SUMO protease. SUMOylation is a reversible post-translational modification that covalently attaches SUMO to target proteins. SUMOylation regulates many critical cellular processes, including replication, cell-cycle, protein transport and DNA repair. SUMO is essential for almost all the eukaryotes, and deregulation of SUMOylation leads to several diseases such as cancer and neurodegenerative diseases. Therefore, it is critical to understand the regulation of SUMO. Capturing and identifying SUMOylated proteins are important to study SUMO pathway. The scientists at Lifesensors have engineered a small peptide, S-Cap, for detection, characterization, and isolation of SUMOylated proteins from cells and tissue extracts.

Applications:

1. The biotin S-Cap can specifically detect SUMOylated protein in combination with HRP-conjugated streptavidin.

2. The biotin S-Cap could be used for isolating SUMOylated protein from cell samples with streptavidin resin.

Benefits:

1. S-Cap has higher specificity and affinity than antibodies

2. Avoid overexpression of epitope-tagged SUMO for pull downs

Components:

Biotin S-Cap (5.9 kDa)

Size: 200 µg

Buffer: 50 mM HEPES, pH 7.5, 150 mM NaCl

Storage: -20°C. Avoid repeated freeze/thaw cycles.

Additional Items Needed:

1. Cell Lysis buffer: 100 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 5 mM EDTA, 1% NP-40, 0.5% Triton-X 100. The use of other buffer systems should not significantly impact S-Cap function; however, the use of alternative detergents (e.g. SDS or deoxycholate) may result in lower recovery efficiency. The inclusion of a general protease inhibitor cocktail is strongly recommended to protect from non-specific protein degradation during lysis and isolation. Also see recommendations 4 and 5 below.
2. Dilution buffer: 100 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 5 mM EDTA
3. Reaction buffer: 100 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 5 mM EDTA, 0.1% NP-40, 0.05% Triton-X 100
   Important: Maintain the concentration of protease inhibitors and S-Cap during this dilution step.
4. Wash buffer: 100 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 5 mM EDTA, 0.05% NP-40.
5. PBS-T: 1x PBS, 0.1% Tween-20
6. TBS-T: 100mM Tris-HCl, pH 8.0, 0.15M NaCl, 5mM EDTA, 0.1% Tween-20
7. N-Ethylmaleimide (NEM), an irreversible inhibitor of all cysteine peptidases.
8. MagnaLink Strepavidin Magnetic Beads (Solulink Cat. No. M-1003-010)

Enrichment of SUMOylated Proteins (Suggested Protocol):

1. Pre-chill cell lysis buffer and microcentrifuge tubes to 4°C. Add NEM (5mM), protease inhibitor cocktail (see manufacturer's instructions), and Biotin S-Cap (0.2 - 2 µM) to the lysis buffer.
   A note on Biotin S-Cap use: Certain factors need to be considered to determine the concentration of this reagent in cell lysates that will ensure detection/enrichment of SUMO. Using the recommended protocol below to pull-down SUMO chains, Biotin S-Cap displays maximal recovery of SUMOylated protein and minimal enrichment of poly-Ubiquitylated proteins between 0.2-2 µM. Therefore, we recommend an initial concentration of 0.2-2 µM Biotin S-Cap (with 50µl of Streptavidin resin) to enrich for SUMOylated proteins. Optimal conditions must be determined by the end user.
2. Wash cells at least 2x with cold PBS. Harvest cells into a centrifuge tube and spin down (~1,000g, 5 min at 4°C). For best results, proceed immediately to cell lysis (Step 3). Cell pellets may be flash frozen and stored in -80°C for later use, without significant loss.
3. Add cold lysis buffer containing Biotin S-Cap and inhibitors to cell pellet. As an initial starting point, we recommend using 100-200µL of lysis buffer for ~1.5×10^6 cells. The optimal number of cells required will depend on cell type and abundance of the protein-of-interest. Resuspend cells in lysis buffer by pipetting or vortexing. Keep all reagents cold during lysis.
4. Clarify lysate by high-speed centrifugation (~14,000g) for 20 min at 4°C.
5. Dilute clarified lysate 5-10-fold in dilution buffer to reduce concentration of NP-40 to 0.1-0.2% and Triton X-100 to 0.05% (Reaction buffer). Adjust the concentration of S-Cap and all inhibitors accordingly.

6. Incubate reaction on ice for 1 to 2h to allow for binding of S-Cap to SUMO chains.
7. Equilibrate magnetic streptavidin resin according to the manufacturer's instructions.
8. Remove an aliquot of "INPUT" sample for comparative analysis by Western blotting.
9. Add cell lysate to equilibrated affinity resin and incubate for 1 to 2 hours at 4°C with gentle rocking or rotation. Additional incubation time may be required; optimal time should be determined by the end user.
10. Collect beads by low-speed centrifugation (~5,000xg, 4ºC) for 5min OR with a magnetic bead stand. Savesupernatant as an "UNBOUND" fraction and prepare an aliquot for comparative analysis with "INPUT" sample.
11. Wash beads with 0.5 to 1ml of cold Wash Buffer, collect by low-speed centrifugation and aspirate thesupernatant carefully to avoid disturbing the beads.
12. Repeat (Step 11) 2-3 times. Useful Tip: Optimization of components in the wash buffer may be required, especially for the isolation of multi-protein complexes.
13. Centrifuge at 13,000g for 3 min to collect the resin OR let it stand on magnetic rack for 3 minutes. Add 10-20ul of 6X SDS reducing sample prep buffer to the resin, and heat at ~95°C for 5 min. The use of reducing agents may result in detection of immunoglobulin light chain components released from the affinity resin depending upon the detection method.
14. Analyze eluted samples by SDS-PAGE/Western blotting in parallel with INPUT and UNBOUND fractions. Discard the resin.

Detection of SUMOylated Proteins by Far-Western (Suggested Protocol):

1. Separate protein samples using a standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protocol.
2. Transfer proteins from the gel to a nitrocellulose/PVDF membrane.
3. Block the membrane using a solution of 5% bovine serum albumin (BSA) in PBS-T buffer at room temperature for 1 hour.
4. Wash the membrane three times for 5 minutes each in TBS-T buffer at room temperature.
5. Incubate the membrane with biotin S-Cap (1:500 – 1:5000 dilution) in TBS-T containing 1% BSA at room temperature with agitation for 2 hours OR overnight.
6. Wash the membrane three times for 5 minutes each in TBS-T at room temperature.
7. Incubate the membrane with HRP-conjugated streptavidin at the recommended concentration in TBS-T containing 1% BSA at room temperature for 1 hour. Adjust the concentration of HRP-conjugated streptavidin to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in TBS-T at room temperature.
9. Develop the blot with ECL reagents.