Biotin SUMO protein capture reagent

Cat. # 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 proline90 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. Here, the team in LifeSensors have developed a small peptide, SUMO protein capture reagent, which specifically recognizes SUMO proteins. This reagent is designed for detection, characterization, and isolation of SUMOylated proteins from cells and tissue extracts.

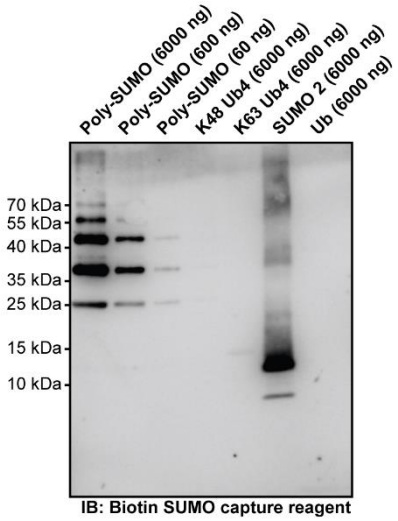
Application:

1. The biotin SUMO protein capture reagent is able to specifically detect SUMOylated protein in combination with HRP-Conjugated Streptavidin.

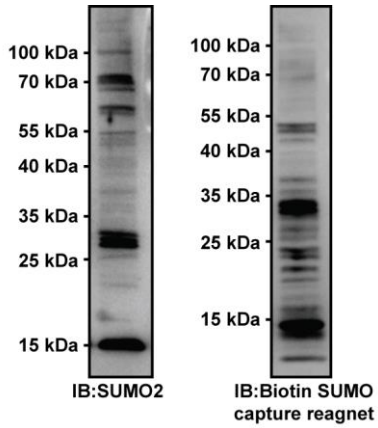
2. The biotin SUMO protein capture reagent could be used for isolating SUMOylated protein from cell samples with Streptavidin resin.

Product Information

Purity:	≥ 90%
Molecular Weight:	5889.42 Da
Supply as:	5 mg/ml in 50 mM HEPES, pH7.5, 150 mM NaCl
Storage:	-20° C. Avoid repeated freeze/thaw cycles



Immunoblot analysis of SUMO and ubiquitn proteins. Biotin SUMO capture reagent was able to detect both poly-SUMO and free SUMO. However, it did not bind to free ubiquitin, or poly-ubiquiitn chain in either K48 or K63 linkage.



Analysis Jurkat cell lysates. 20 µg of cell lysate was applied to immunoblot. Both SUMO2 antibody and biotin SUMO capture reagent were used to probe the sumolyated proteins.

Procedure

Immunoblotting

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 3% bovine serum albumin (BSA) in PBST at room temperature for 1 hour.
4. Wash the membrane three times for 5 minutes each in TBST at room temperature.
5. Incubate the membrane with biotin SUMO protein capture reagent (1:500 – 1:5000 dilution) in TBST containing 1% BSA at room temperature with agitation for 2 hours or overnight.
6. Wash the membrane three times for 5 minutes each in TBST at room temperature.
7. Incubate the membrane with peroxidase-conjugated streptavidin at the recommended concentration in TBST containing 1% BSA at room temperature for 1 hour. Adjust the peroxidase-conjugated streptavidin concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in TBST at room temperature.
9. Treat the membrane with a peroxidase substrate.

Pull-down

SUMO protein capture reagent can be used in pull-down procedure when used in conjugation with streptavidin beads.

1. Take the desired amount of biotin SUMO protein capture reagent and incubate with streptavidin beads for 30 minutes.
2. Wash the beads with PBST for 3 times.
3. Apply the biotin SUMO protein capture reagent conjugated beads to the cell lysate, incubating at 4 ºC for 2 hours.
4. Wash the beads with PBST for 3 times.
5. Add SDS-PAGE sample buffer and analyze the sample by immunoblot.

References

1. Eifler K, Vertegaal AC: **Mapping the SUMOylated landscape**. *FEBS J* 2015, **282**(19):3669-3680.