V.5.0

SUMO-2/3 Affinity Beads

Cat. # ASM24-Beads

Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	2 x 400 µl when reconstituted
Validated applications:	IP
Species reactivity:	Broad reactivity
Host/Isotype:	Mouse/IgG1-kappa
Clone:	11G2

Background Information

Small Ubiquitin-related Modifiers (SUMOs) are 12 kDa post translational modification (PTM) proteins that are highly conserved from yeast to mammalian cells[1]. In budding yeast (*Saccharomyces cerevisiae*), only one SUMO protein (Smt3) exists, in vertebrates, three major SUMO isoforms (SUMO-1, SUMO-2, and SUMO-3) are expressed in all tissue[2]. SUMO-1 is also known as SMT3C, Sentrin, GMP1, UBL1 and PIC1. Mature SUMO-2 and SUMO-3 are 97% identical in amino acid sequence (48% identity with SUMO-1) and appear to be functionally identical. SUMO proteins (SUMO-1 vs SUMO-2/3) show distinct sub-cellular localization, also the expression level of SUMO-2/3 is generally higher than that of SUMO-1[3-5]. Proteins are post translationally modified by SUMO conjugation (SUMOylation) to an acceptor lysine residue within a target protein consensus sequence ψKXE (where ψ represents a hydrophobic amino acid and X represents any amino acid). While only a single SUMO-1 is ligated to target proteins, SUMO-2/3 proteins form poly-SUMO-2/3 chains on target proteins that can be terminated by SUMO-1 ligation[6,7]. SUMOylation is a highly dynamic reversible PTM that has been demonstrated to be involved in a diverse array of cellular processes, including regulation of gene expression, protein stability, protein transport, mitosis and protein-protein interaction[8,9].

Material

SUMO-2/3 affinity beads consist of the anti-SUMO-2/3 antibody (clone: 11G2) that has been chemically conjugated to Protein G beads. The affinity bead has been shown to immunoprecipitate a wide range of SUMO-2/3 targeted proteins in HeLa cell lysate (Fig. 1A). The bead reagent has been optimized to give no detectable leaching of either heavy or light chains in an IP assay, making the resulting data extremely specific, sensitive and clean (Fig. 1A & 1B).
Clone 11G2 is a mouse monoclonal antibody. The antibody was raised against full-length recombinant SUMO-2 protein (Uniprot: P61956) combined with a proprietary mix of peptides that include CQIRFRFDGQPINE. The linear epitope has not been identified and seems recognize a conformational epitope. ASM24-Beads are supplied as a lyophilized powder.

Storage and Reconstitution

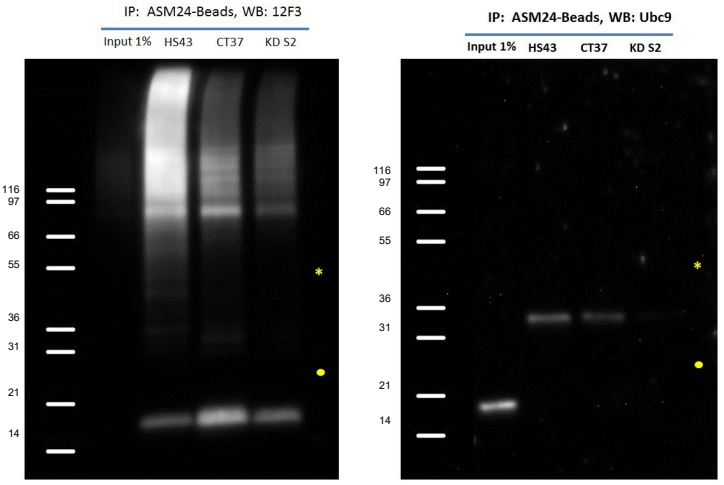
Shipped at ambient temperature. The lyophilized beads can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder to the bottom of the tube. Reconstitute each tube in 400 µl of 50% glycerol in water and store at –20°C. Final buffer composition is 200mM PIPES pH 7.4, 5% sucrose, 50% glycerol and 1% dextran. When stored and reconstituted as described, the product is stable for 6 months at 4°C. NOTE: We recommend adding an antibacterial such as sodium azide (0.02% final concentration) to prevent bacterial contamination of the antibody-bead stock.

Applications

Immunoprecipitation (IP) Applications

The working concentration should be determined by users empirically. IP performance of ASM24-Beads has been confirmed using 40ul of ASM24-Beads (50% bead slurry) to immunoprecipitate SUMO 2/3 target proteins and free SUMO 2/3 from 1mg of cell lysates.

Figure 1: IP of HeLa cells with ASM24-Beads



Legend: Denatured cell lysates were prepared as previously reported[10] from HS43, CT37 and KD S2 (HS43: Heat Shock treated (43°C for 10min), CT37: untreated and KD S2: shRNA SUMO -2 knock down). 1mg of lysate was used for the immunoprecipitation of SUMO-2/3 conjugates. IP experiments were performed by the protocol presented in IP and WB Method. Western blots of immunoprecipitated proteins were developed using anti-SUMO-2/3 antibody (Cytoskeleton cat# ASM23) (A) or anti-Ubc9 antibody (B). The level of SUMO-2/3 conjugates in heat shock treated cells is higher than control and shRNA SUMO-2 knock-down reduced the level of the conjugates. Chemical conjugation of 11G2 to agarose beads prevents heavy and light chain leaching completely. Star (*) and circle (o) indicate positions of heavy and light chains of antibodies. Unconjugated free SUMO is denoted by triangle. (B) Unconjugated Ubc9 is visible near 120kDa. High molecular-weight band indicates that Ubc9 is conjugated by single SUMO-2/3 protein. Ubc9 has previously been reported to be a target for Sumoylation [10,11].

IP and WB Method

1. Incubate 40ul of 11G2 conjugated beads (Cat# ASM24-Beads) with 1mg of cell lysate (0.5mg/ml) for 2 hr or overnight if convenient at 4°C with a rotation.
2. Wash beads 3 times by a centrifugation (960 x g, 4°C, 1 min) and resuspension with washing buffer (50mM Tris pH7.5, 150mM NaCl, 1% IGEPAL, 20mM NEM and protease inhibitor (e.g. Cytoskeleton cat# PICO2)).
3. Resuspend beads in 30 µl of 2X non-reducing SDS sample buffer (125mM Tris pH6.8, 20% glycerol, 4% SDS, 0.005% Bromophenol blue).
4. Incubate the solution at room temperature for 5min and collect supernatant (~30ul) after centrifugation (960 x g, 1 min., room temp).
5. Add 1ul of beta mercaptoethanol to each sample and boil for 5 min prior to loading on SDS-PAGE.

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6. Run protein samples and control sample (IP input) in SDS-PAGE.
7. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, and 15% methanol) for 15 min at room temperature prior to electro-blotting.
8. Transfer the protein to a PVDF membrane overnight (10-18h) at constant 20 V.
9. Wash the membrane once with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
10. The membrane may be left in TBST overnight at 4°C if convenient.
11. Block the membrane surface with 5% nonfat-dry milk in TBST for 30 min at roomtemperature with constant agitation.
12. Incubate the membrane with a primary antibody (e.g. For SUMO-2/3 use Cytoskeleton cat# ASM23) for 1-2 h at room temperature or overnight at 4°C with constant agitation.
13. Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
14. Incubate the membrane with an appropriate dilution (e.g., 1:20,000) of anti-mouse secondary antibody (e.g., goat anti-mouse HRP conjugated IgG from Jackson Labs., Cat. # 115-035-068) in TBST for 60 min shaking at room temperature.
15. Wash the membrane 4 times in TBST for 10 min each.
16. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

References

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