Anti-SUMO1 (7A1A2 mAb) Affinity Beads

Cat. # ASM11-bead

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

Form:	Lyophilized powder
Amount of material:	3 x 400 µl when reconstituted
Validated applications:	IP
Species reactivity:	All
Host/Isotype:	Mouse/ IgG2b
Clone:	7A1A2

Background Information

In mammalian cells, the small ubiquitin-like modifier (SUMO) family contains four isoforms (SUMO1, SUMO2, SUMO3, and SUMO4), and functions as a 12 kDa post-translational modification (PTM) protein. SUMO2 and SUMO3 are nearly identical, differing in only three amino acid residues. SUMO1, also known as SMT3C, Sentrin, GMP1, UBL1, and PIC1, shares 48% identity with SUMO2/3 (1). SUMO4 is about 85% identical to SUMO2/3, but it is unclear whether SUMO4 can be conjugated to substrates (2). 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). Similar to ubiquitination, SUMOylation requires a three enzymes system (E1, E2, and E3) to conjugate SUMO covalently to target substrates. The covalently linked SUMO can be removed by sentrin-specific proteases (SENPs), a process known as deSUMOylation (3). SUMOylation is a highly dynamic, reversible PTM (PTM) that regulates the activity, subcellular localization, stability, and functions of target proteins and thereby modulates almost all major cellular pathways (4).

Material

ASM11 anti-SUMO1 affinity beads are composed of our newly developed anti-SUMO1 antibody clone 7A1A2 (IgG2b), that has been covalently crosslinked to protein G beads. ASM11-beads enriched a more complete profile of SUMO1 modified proteins from HAP1 wildtype cells while also minimizing non-specific interactions (see SUMO1 knockout [KO] lane) versus other SUMO1 affinity reagents (Fig 1). Importantly, conjugated beads significantly minimize heavy and light chain leaching (Fig 1). Enhanced enrichment of target proteins are especially important when investigating target protein SUMO1 modification of less abundant proteins (Fig 2). ASM11 affinity beads are supplied as a lyophilized white powder.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized affinity beads can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube.

Reconstitute each tube in 400µl of 50% glycerol in water (room temperature). Make sure beads are completely hydrated before use. Store reconstituted affinity beads at -20°C. Final buffer composition is 200 mM PIPES, 50% glycerol, 5% sucrose, and 1% dextran.

When stored and reconstituted as described, the product is stable for 12 months at -20°C. NOTE: We recommend adding an antibacterial such as sodium azide (0.02% final concentration) to prevent bacterial contamination of the antibody stock.

Immunoprecipitation (IP) Applications

Use as indicated at 40 µl bead slurry per IP reaction (1mg total lysate per IP), sufficient for approximately 30 IP assays.

IP Method

1. Briefly mix bead by vortexing. Make sure bead is in suspension.
2. Transfer 40 µl of affinity bead slurry to 1ml of PBST in a microfuge tube with an end snipped pipet tip. Mix well and spin down beads for 1 min at 4°C and 3000 rpm.
3. Repeat PBST wash one more time.
4. Add 1-1.5 mg of cell lysate (1-1.5 mg/ml protein concentration) to the beads. The lysate must be prepared in an IP compatible buffer (e.g. BlastR lysis buffer and filter system).
5. Gently rotate the reaction at 4°C for 2 h.
6. Spin down beads for 1 min 4°C at 3000 rpm (approx. 960 x g).
7. Discard supernatant and wash beads with 1ml of IP wash buffer (e.g BlastR wash buffer) at 4°C.
8. Repeat wash two more times.
9. Add 30 µl of 2X **non-reducing** Laemmli buffer (125 mM Tris pH 6.8, 20% glycerol, 4% SDS, and 0.005% Bromophenol blue), mix beads well and incubate at room temp for 5 minutes.
10. Spin down beads at 3000 rpm for 1 minute at 4°C. Carefully transfer supernatant, without disturbing beads, to a new microtube containing 1ul of β-mercaptoethanol. Boil for 5 min prior to loading on SDS-PAGE for subsequent Western blot analysis.
11. Example IP data is shown in Figs. 1 & 2. Control beads (Cat # CIG03) are included to eliminate any non-specific binding to beads. Lanes 21C7 and D11 Fig.1 and 21C7 (Fig.2) show IP data obtained from other commercially available SUMO1 antibody/beads.

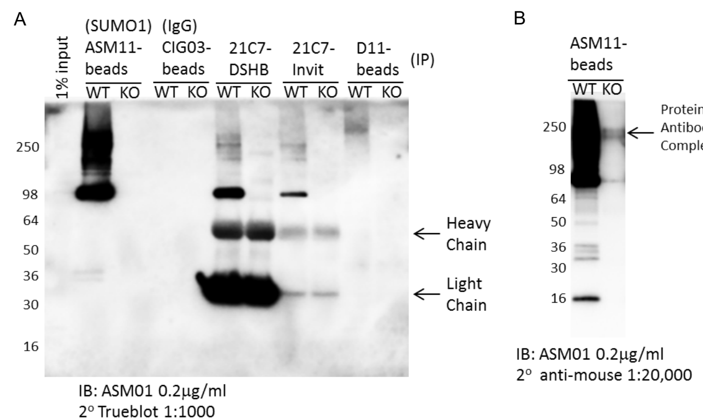

Fig 1: **Total SUMO1 profiles.** (A) HAP1 wildtype (WT) or SUMO1 knockout (KO) lysate, was obtained using BlastR lysis and filter system. 1 mg of each lysate were incubated with 40 µg of each SUMO1 affinity reagent: ASM11-beads (Cytoskeleton), 21C7 (Invitrogen—purified), 21C7 (DSHB—supernatant), D11-beads (Santa Cruz) and conjugated SUMO1 IgG control beads (CIG03). 21C7 antibodies were captured with protein G agarose beads to enrich for SUMO-1 modified proteins. Samples were separated by SDS-PAGE and transferred to PVDF. Enriched SUMO1 samples were analyzed by western blot using ASM01 (Cytoskeleton) antibody at 1:5000, and mouse Trubelot Ultra-HRP secondary at 1:1000 in 5% milk. Trueblot secondary was used to minimize heavy and light chain detection from 21C7 samples. (B): IP was performed using ASM11 as in Fig 1A. SUMO1 modified proteins were visualized with ASM01 1:5000, and anti-mouse secondary at 1:20,000 to highlight the profile of SUMOylated proteins in the region between 64-30 kDa that may be masked by heavy and light chain interference when using unconjugated antibodies for IP.

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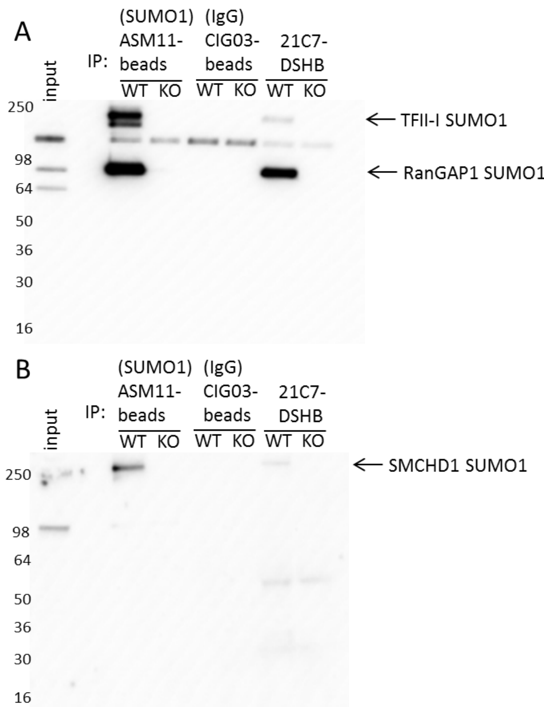

Fig 2: **SUMO1 modified target proteins.** HAP1 wildtype (WT) or SUMO1 knockout (KO) lysate, was obtained using BlastR lysis and filter system. 1 mg of each lysate were incubated with 40 μg of each SUMO1 affinity reagent: ASM11-beads (Cytoskeleton), 21C7 (DSHB—supernatant), and conjugated SUMO1 IgG control beads (CIG03). 21C7 antibodies were captured with protein G agarose beads to enrich for SUMO-1 modified proteins. Samples were separated by SDS-PAGE and transferred to PVDF. Target proteins: (A) TFII-I, RanGAP1, and (B) schmd1 were analyzed for their SUMO1 modified forms by western blot. Anti-rabbit-HRP labeled secondary antibody was used at 1:10,000. All three primary antibodies are rabbit polyclonal antibodies, and should not bind heavy and light chain fragments from the IP antibody.

References

1. Guo C. and Henley J. 2014. Wrestling with stress: Roles of protein SUMOylation and de-SUMOylation in cell stress response. IUBMB Life. 66, 71-77.
2. Owerbach D. et al. 2005. A proline-90 residue unique to SUMO-4 prevents maturation and sumoylation. Biochem. Biophys. Res. Comm. 337, 517-520.
3. Yang W. and Paschen W. 2015. SUMO proteomics to decipher the SUMO-modified proteome regulated by various diseases. Proteomics. 15, 1181-1191.
4. 4. Kira B. et al. 2012. SUMOylation in carcinogenesis. Cancer Lett. 316, 113-125.

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