V.1.4

Anti-SUMO 1 Mouse Monoclonal Antibody

Cat. # ASM01

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

Online Datasheet Contains ASM01 (V1.4) and ASM01-S (V1.4)

Form:	Lyophilized powder
Amount of material:	1 x 100 µl when reconstituted
Validated applications:	WB, IP
Species reactivity:	All
Host/Isotype:	Mouse/IgG2b
Clone:	5D8B16

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

Anti-SUMO1 antibody ASM01 is a mouse monoclonal antibody. The antibody was raised against full-length recombinant SUMO1 protein (Uniprot: P63165). The antibody has been shown to recognize a broad profile of SUMO1 proteins in HAP1, HeLa, and 293 cell lysates (Fig. 1A) and to detect sub-nanogram amounts of recombinant SUMO1 (Fig. 1B). The antibody shows high specificity (Fig. 1A WT vs KO lane) with no cross reactivity to SUMO2 (Fig. 1B). Furthermore, the sensitivity of ASM01 was compared to commercially available SUMO1 antibodies, and the data shows that ASM01 detects a more robust and specific profile of SUMO1 modified proteins (Fig. 1C). ASM01 is purified by Protein G affinity chromatography and is supplied as a lyophilized white powder.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized antibody 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 100ul of 50% glycerol in water (room temperature). We do not recommend using 50% glycerol solution at 4°C as this can cause the lyophilized antibody to stick to the pipet tip during resuspension. Store reconstituted antibody at –20°C. Final buffer composition is 200 mM PIPES, 50% glycerol, 5% sucrose, and1% 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.

Applications

Western Blot (WB) Applications

Use as indicated below at 1:5000 dilution, sufficient for 500 ml of working strength antibody.

Western Blot Method:

1. Run protein samples and control samples in SDS-PAGE.
2. We recommend running 10 µg of control cell lysate. (e.g. HAP1 wildtype and knockout cell lysate, or lysate harvested +/- NEM).
3. 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.
4. Transfer the protein to a PVDF membrane for 60 min at 70 V.
5. Wash the membrane once with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
6. The membrane may be left in TBST overnight at 4°C if convenient.
7. Block the membrane surface with 5% nonfat-dry milk in TBST for 60 min at room temperature with constant agitation.
8. Incubate the membrane with a 1:5000 dilution of anti-SUMO1 antibody, diluted in 5% nonfat-dry milk in TBST, for 1-2 h at room temperature or overnight at 4°C with constant agitation.
9. Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
10. 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/5% non-fat milk for 60 min shaking at room temp.
11. Wash the membrane 5 times in TBST for 10 min each.
12. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

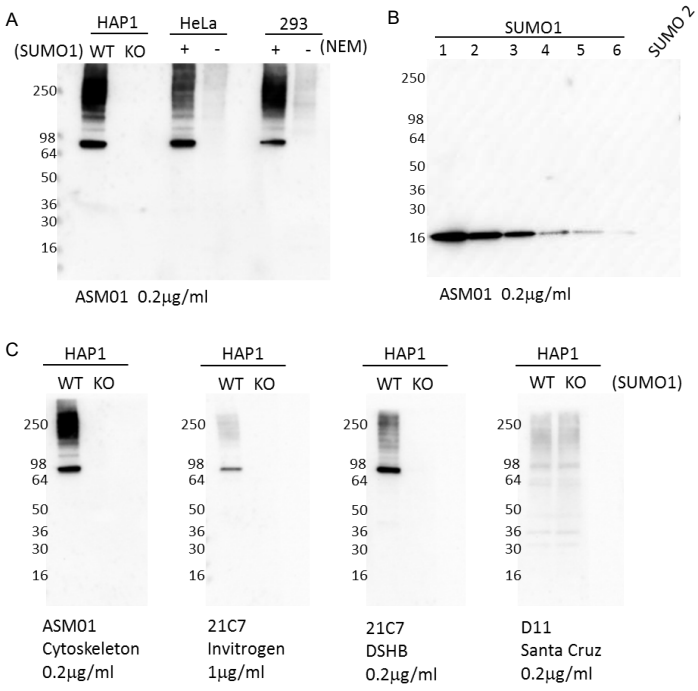

Fig 1: ASM01 was used at a 1:5000 dilution (0.2 µg/mL) following the recommended western blot protocol (see above). Figure 1A: HAP1 wildtype (WT) or SUMO1 knockout (KO) lysate, HeLa cell +/- NEM lysate and 293 cell +/- NEM lysate was obtained using BlastR lysis and filter system. 10 µg of each lysate were separated by SDS-PAGE and transferred to PVDF. Robust SUMO1 profiles were detected for every cell type. Specificity is shown by the lack of SUMO1 detection in SUMO1 KO cells and significantly diminished profiles in lysates prepared in the absence of the SENP inhibitor (NEM). Figure 1B: Titration of recombinant SUMO1 lanes 1-6 contain 5.0, 2.5, 1.25, 0.6, 0.3, and 0.15 ng SUMO1, while lane 7 contains 1000 ng of recombinant SUMO2. Figure 1C: HAP1 WT and KO lysate as prepared in 1A was used. 10 µg of each lysate were separated by SDS-PAGE and transferred to PVDF. SUMO1 proteins were detected using the recommended concentrations of ASM01 (Cytoskeleton), 21C7 (Invitrogen—purified), 21C7 (DSHB—supernatant), and D11 (Santa Cruz) as shown in the figure. All samples were developed and imaged simultaneously to ensure identical experimental conditions.

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Immunoprecipitation (IP) Applications

Use as indicated at 40 µl per IP reaction, sufficient for approximately 2.5 IP assays. ASM01 is effective for IP applications and captures a robust profile of SUMO1 modified proteins, and outperforms currently available SUMO1 antibodies at enriching SUMO1 modified proteins (Fig. 2).

Note: It is recommended to use ASM11-beads for SUMO1 IP assays for maximal sensitivity, cost efficiency, and to minimize antibody heavy and light chain interference (Fig 2).

IP Method

1. Add 40 µl of antibody to 500 µl of PBS pH 7.4 in a microfuge tube containing 20 µl of packed Protein G agarose pre-equilibrated in PBS.
2. Gently rotate the reaction for 1 h at 4°C.
3. Add 500 µl of PBST to the mixture and centrifuge for 1 min at 4°C and 3000 rpm (approx. 960 x g). Addition of the PBST will prevent agarose from sticking to the microfuge tube walls.
4. Discard supernatant and wash beads 3X in PBST.
5. 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).
6. Gently rotate the reaction at 4°C for 2 h.
7. Spin down agarose for 1 min 4°C at 3000 rpm (approx. 960 x g).
8. Discard supernatant and wash beads with 1ml of IP wash buffer (e.g BlastR wash buffer) at 4°C.
9. Repeat wash two more times.
10. Resuspend beads in 30 µl of 2X Laemmli buffer (125 mM Tris pH 6.8, 20% glycerol, 4% SDS, 0.005% Bromophenol blue, 5% beta-mercaptoethanol) and boil for 5 min prior to loading on SDS-PAGE for subsequent Western blot analysis.

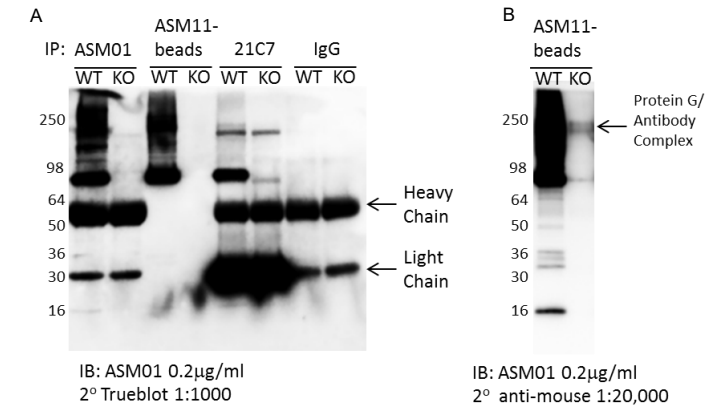

Fig 2: HAP1 wildtype (WT) and knockout (KO) cells were prepared in BlastR buffer and filter system supplemented with NEM and PIC02. Figure 2A: 1 mg of lysate per reaction was used to immunoprecipitate (IP) SUMO1 modified proteins using 40µg of 5D8 antibody (ASM01), 40µg of ASM11-beads, 40µg of 21C7 (DSHB-supernatant), or 40µg of mouse IgG control antibody. 5D8, 21C7 supernatant, and mouse IgG were bound to Protein G Agarose as described in the method (see above). Enriched SUMO1 samples were analyzed by western blot using ASM01 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. Figure 2B: IP was performed using ASM11 as in Fig 2A. 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.

Immunofluorescence (IF) Applications

ASM01 has not been tested for this application.

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.

Product Citations/Related Products

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V.1.4

Anti-SUMO 1 Mouse Monoclonal Antibody

Cat. # ASM01-S

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

Form:	Lyophilized powder
Amount of material:	1 x 25 µl when reconstituted
Validated applications:	WB, IP
Species reactivity:	All
Host/Isotype:	Mouse/IgG2b
Clone:	5D8B16

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).

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Storage and Reconstitution
Shipped at ambient temperature. The lyophilized antibody 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 25ul of 50% glycerol in water (room temperature). We do not recommend using 50% glycerol solution at 4°C as this can cause the lyophilized antibody to stick to the pipet tip during resuspension. Store reconstituted antibody at –20° C. Final buffer composition is 200 mM PIPES, 50% glycerol, 5% sucrose, and1% 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.

Applications
Western Blot (WB) Applications
Use as indicated below at 1:5000 dilution, sufficient for 125 ml of working strength antibody.

Western Blot Method:
1. Run protein samples and control samples in SDS-PAGE.
2. We recommend running 10 µg of control cell lysate. (e.g. HAP1 wildtype and knockout cell lysate, or lysate harvested +/- NEM).
3. 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.
4. Transfer the protein to a PVDF membrane for 60 min at 70 V.
5. Wash the membrane once with TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
6. The membrane may be left in TBST overnight at 4°C if convenient.
7. Block the membrane surface with 5% nonfat-dry milk in TBST for 60 min at room temperature with constant agitation.
8. Incubate the membrane with a 1:5000 dilution of anti-SUMO1 antibody, diluted in 5% nonfat-dry milk in TBST, for 1-2 h at room temperature or overnight at 4°C with constant agitation.
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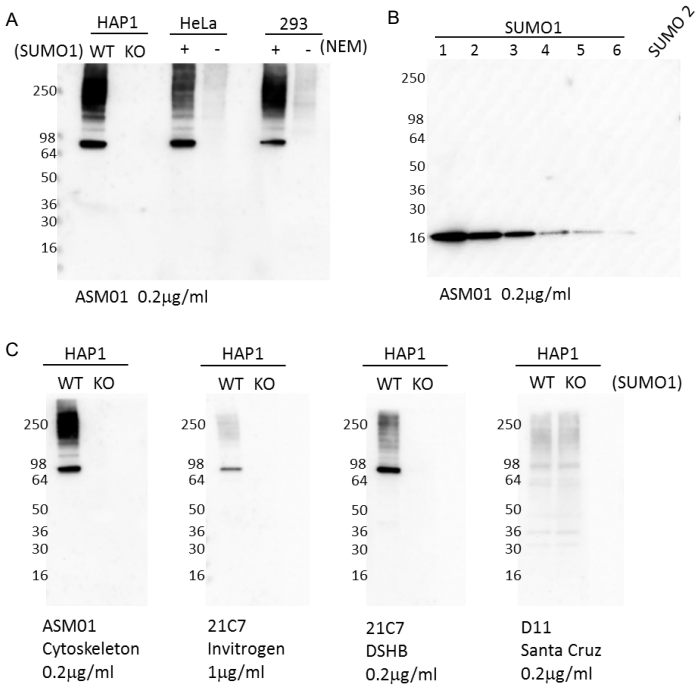

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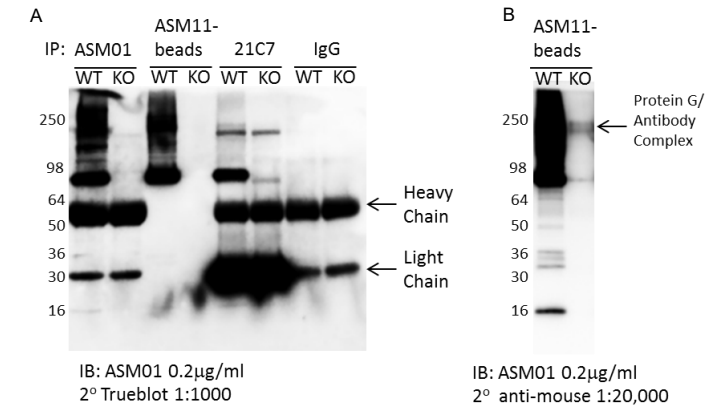

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