

Anti-SUMO-2/3 Mouse Monoclonal Antibody

Cat. # ASM23-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, IF and IP
Species reactivity:	Broad reactivity
Host/Isotype:	Mouse/IgG2a-kappa
Clone:	12F3

Background Information

Small Ubiquitin-related Modifiers (SUMOs) are 12 kDa post-translational modification (PTM) proteins that are highly conserved from yeast to mammalian cells¹. 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 tissue². 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 and the expression level of SUMO-2/3 is generally higher than that of SUMO-1³⁻⁵. Proteins are post-translationally modified by SUMO conjugation (SUMOylation) to an acceptor lysine residue within a target protein consensus sequence ψ KXE (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

Anti-SUMO-2/3 antibody 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 antibody has been shown to recognize a wide range of SUMO-2/3-targeted proteins in HeLa cell lysate (Fig. 1B) and to detect sub-nanogram amounts of recombinant SUMO-2 (Fig. 1A). Epitope mapping has identified that the antibody recognizes a sequence/structure within the peptide CQIRFRFDGQPINE. The peptide sequence is conserved in mammals, birds, and amphibians, giving the antibody broad species reactivity. ASM23 is purified by Protein G affinity chromatography and is supplied as a lyophilized white powder.

Storage and Reconstitution

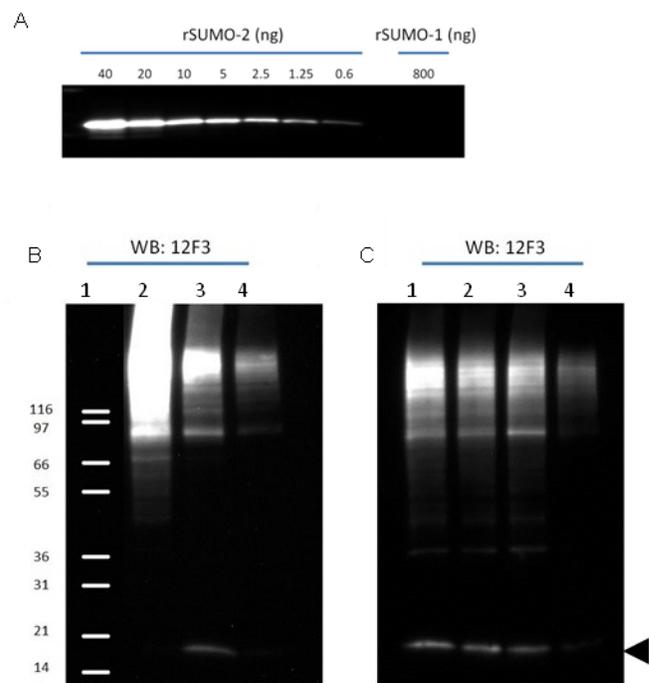
Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder to the bottom of the tube. Reconstitute in 25 µl of water and store at 4°C. **DO NOT FREEZE**. Final buffer composition is 200 mM PIPES pH 7.4, 1% sucrose, and 0.5% 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 stock.

Applications

Western Blot (WB) Applications

Use as indicated below at 1:500-1:1000 dilution, sufficient for 12.5-25 ml of working strength Ab.

Figure 1 : Western blot applications



Legend: 12F3 was used for immuno-blotting (1:500 dilution) following the recommended Western blot protocol (see below). **Figure 1A:** Titrations of recombinant SUMO-2 (40-0.6 ng) and SUMO-1 (800 ng). SUMO-2 was detected down to 0.6 ng while SUMO-1 was not detected at 800 ng. **Figure 1B:** Induction of SUMOylation by heat shock and reduction of SUMOylation by SUMO-2 shRNA knockdown. Cell lysates were prepared from HeLa cells: Lane 2: Heat Shock treated (43°C for 10min), Lane 3: untreated, Lane 4: shRNA SUMO-2 knock down. 20µg of HeLa cell lysates were used for each sample. Lane 1: position of molecular weight markers. **Figure 1C:** Specificity of SUMO-2 knockdown signal. Lane 1: parental HeLa cell lysates, Lane 2: SUMO-2 shRNA control lysates, Lane 3: SUMO-1 shRNA knock-down cell lysates, Lane 4: SUMO-2 shRNA knock-down cell lysates. Arrow head indicates free SUMO-2/3.

Western Blot Method:

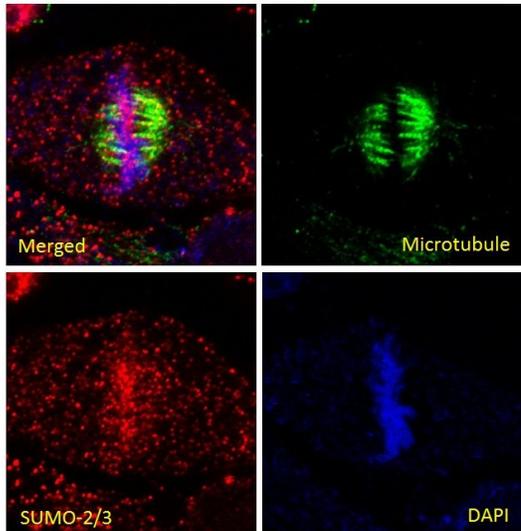
1. Run protein samples and control samples in SDS-PAGE.
2. We recommend running 20 µg of HeLa cell lysate as a control.
3. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, and 15% methanol) for 15 min at room temperature prior to electro-blotting.
4. Transfer the protein to a PVDF membrane overnight at constant 20 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 30 min at room

- temperature with constant agitation.
- Incubate the membrane with a 1:500-1:1000 dilution of anti-SUMO-2/3 antibody, diluted in TBST, for 1-2 h at room temperature or overnight at 4°C with constant agitation.
- Rinse the membrane three times in 50 ml TBST for 10 min. each at room temperature with constant agitation.
- 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.
- Wash the membrane 4 times in TBST for 10 min each.
- Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

Immunofluorescence (IF) Applications

Use as indicated below at 1:500 dilution, sufficient for 12.5 ml of working strength Ab, approx. 12 IF staining.

Figure 2: IF of HeLa cells in metaphase with ASM23 antibody (clone 12F3)



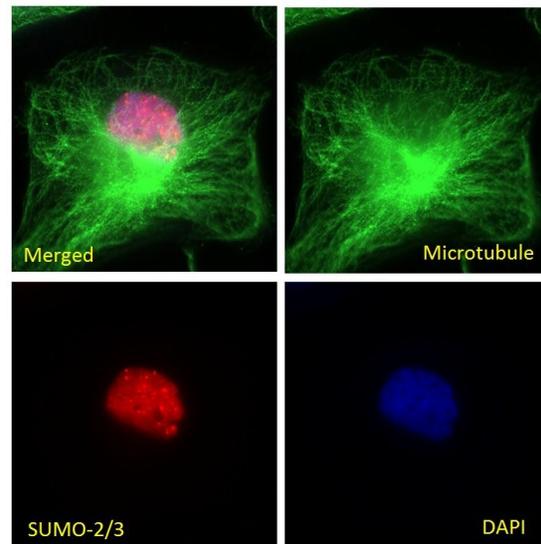
Legend: HeLa cells were stained and visualized by confocal fluorescence microscopy as described in the IF method below. The cells were stained against α/β -tubulin (sheep anti-tubulin Ab, Cat# ATN02, green) and SUMO-2/3 (12F3, red). DNA was stained with DAPI. Mitotic cells in metaphase were imaged with a Zeiss LSM 780 confocal microscope (1.4 NA 63X objective). Enrichment of SUMO 2/3 at chromosomes can be observed during mitosis as has been previously reported¹⁰.

IF Method for Mitotic Cells

- Plate HeLa cells at 3×10^5 /ml in glass bottom dish (MatTek cat# P35G-1.5-14-C) with DMEM media containing 10% FBS.
- Allow cells to grow for 24-48 h to reach 80% confluency.
- Permeabilize cells by incubating them with 3ml of digitonin solution (20mM HEPES pH 7.4, 110 mM Potassium acetate, 2 mM Magnesium acetate, 1X protease inhibitor cocktail, 10 mM NEM and 40 ug/mL digitonin) for 1 min with gentle agitation at room temperature. NOTE: we have found the timing of digitonin treatment and gentle agitation to be critical to successful chromosomal localization of SUMO2/3.
- Wash the cell plate with PBS briefly. Add 3 ml of 4% paraformaldehyde solution and incubate for 10min at room temperature.
- Wash the cell plate two times with PBS (10 min incubation per wash, no agitation).
- (Optional) Incubate in 3ml of blocking solution (e.g. PBS containing 3% BSA) for 30 min. Aspirate blocking solution.
- Apply 1 ml of 12F3 solution (1:500 in PBS) containing other probing reagent (e.g. anti-tubulin antibody and DAPI).
- Incubate at room temperature for 45 min.
- Wash the cell plate two times with PBS (10 min incubation per wash, no agitation).

- Apply 1 ml of fluorescently-labeled secondary antibody solution at manufacturer's recommended dilution. For example, we use fluorescently-labeled donkey anti-mouse at 1:500 dilution in PBS to visualize SUMO-2/3 (red) and donkey anti-sheep at 1:500 to visualize microtubules (green) (Fig. 2).
- Incubate at room temperature for 45 min.
- Wash the cell plate two times with PBS over 20 min.
- Observe cells under fluorescence microscope.

Figure 3: IF of HeLa cells in interphase with ASM23 antibody (clone 12F3)



Legend: HeLa cells were stained and visualized by widefield fluorescence microscopy as described in the IF method below. The cells were stained against α/β -tubulin (sheep anti-tubulin Ab, Cat# ATN02, green) and SUMO-2/3 (12F3, red). DNA was stained with DAPI. Cells in interphase were imaged with a Zeiss Axio Observer.Z1 microscope (1.4 NA 63X objective). PML nuclear bodies (nuclear dots) were visible in SUMO-2/3 staining as has been previously reported¹¹.

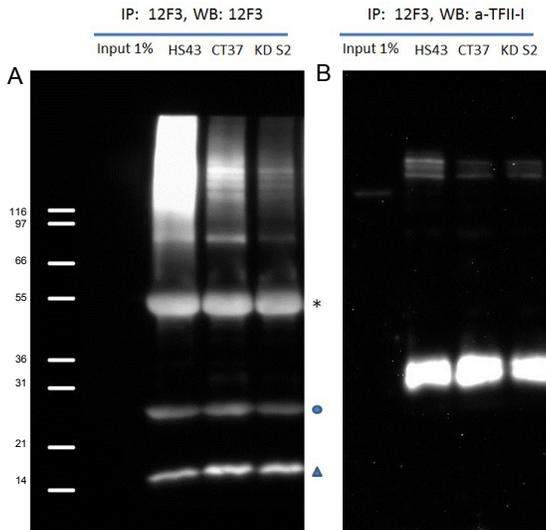
IF Method for non-mitotic cells

- Plate HeLa cells at 3×10^5 /ml in glass bottom dish (MatTek cat# P35G-1.5-14-C) with DMEM media containing 10% FBS.
- Allow cells to grow for 24-48 h to reach 80% confluency.
- Fix cells by adding 3ml of 4% paraformaldehyde solution and by incubating for 10min at room temperature.
- Wash the cell plate with PBS briefly (2 minute incubation, no agitation).
- Permeabilize cells by incubating in 3ml of 0.5% Triton X-100 solution for 5 min at room temperature.
- Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
- (Optional) Incubate in 3ml of blocking solution (e.g. PBS containing 3% BSA) for 30 min. Aspirate blocking solution.
- Apply 1ml of 12F3 solution (1:500 in PBS) containing other probing reagent (e.g. anti-tubulin antibody and DAPI).
- Incubate at room temperature for 45 min.
- Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
- Apply 1ml of fluorescently-labeled secondary antibody solution at manufacturer's recommended dilution. For example, we use fluorescently-labeled donkey anti-mouse at 1:500 dilution in PBS to visualize SUMO-2/3 (red) and donkey anti-sheep at 1:500 to visualize microtubules (green) (Fig. 3).
- Incubate at room temperature for 45 min.
- Wash the cell plate two times with PBS (10 min incubation per wash, no agitation).
- Observe cells under fluorescence microscope.

Immunoprecipitation (IP) Applications

Working concentration should be determined by users empirically. IP performance of 12F3 has been confirmed using 30ul of 12F3 (Cat# ASM23) to IP SUMO 2/3 conjugated proteins and free SUMO 2/3 from 1mg of cell lysates.

Figure 4: IP of HeLa cells with ASM23 antibody



Legend: Denatured cell lysates were prepared from HS43, CT37 and KD S2¹². 1mg of lysate was used for the immunoprecipitation of SUMO-2/3 conjugates. IP experiments were performed by the protocol presented in IP Method. Western blots of immunoprecipitated proteins were developed using 12F3 (A) or anti-TFII-I antibody (B). (A) Star (*) and circle (o) indicate heavy and light chains of antibodies. Unconjugated free SUMO is denoted by triangle. (B) Unconjugated TFII-I is visible near 120kDa. Multiple bands indicate that TFII-I is SUMOylated by several SUMO-2/3 proteins. TFII-I has previously been reported to be a target for Sumoylation^{12,13}.

IP Method

1. Incubate 30 μ l of 12F3 (Cat# ASM23) with 1mg of cell lysate (0.5 mg/ml) for 1 hr in ice.
2. Add 30ul of Protein G slurry and incubate for 2 hr or overnight if convenient at 4°C with a rotation.
3. 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).
4. Resuspend beads in 30 μ l of 2X non-reducing SDS sample buffer (125 mM Tris pH6.8, 20% glycerol, 4% SDS, 0.005% Bromophenol blue).
5. Incubate the solution at room temperature for 5min and collect supernatant (~30 μ l) after centrifugation (960 x g, 1 min., room temp).
6. Boil the sample with 1 μ l of beta mercaptoethanol for 5 min prior to loading on SDS-PAGE for subsequent Western blot analysis.

References

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