V.1.1

# Anti-SUMO-2/3 Mouse Monoclonal Antibody

**Cat. # ASM23**

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

| | |
|---|---|
| **Form:** | Lyophilized powder |
| **Amount of material:** | 2 x 100 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 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 and 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

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.

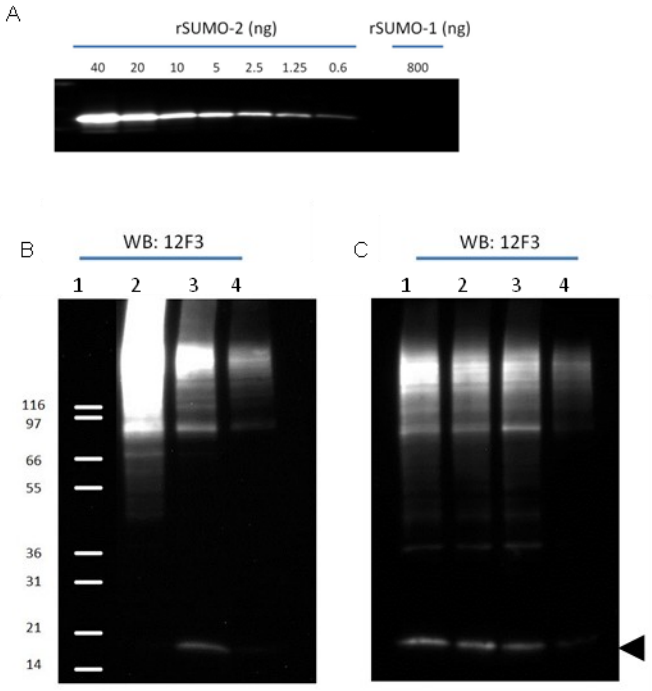
## Storage and Reconstitution

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 each tube in 100 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 100-200 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

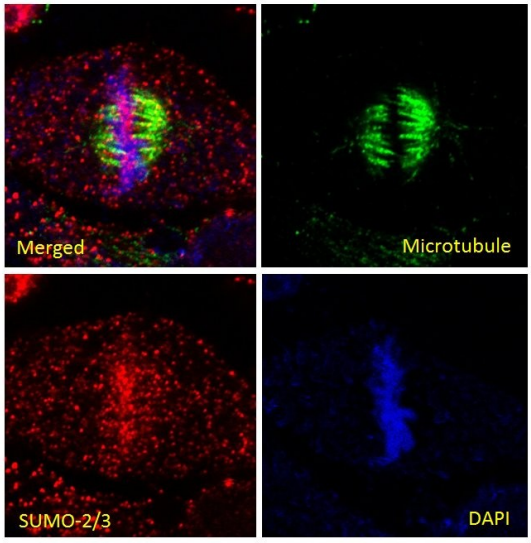
temperature with constant agitation.

8. 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.
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 for 60 min shaking at room temperature.
11. Wash the membrane 4 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).

**Immunofluorescence (IF) Applications**

Use as indicated below at 1:500 dilution, sufficient for 100 ml of working strength Ab, approx. 100 IF staining.

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

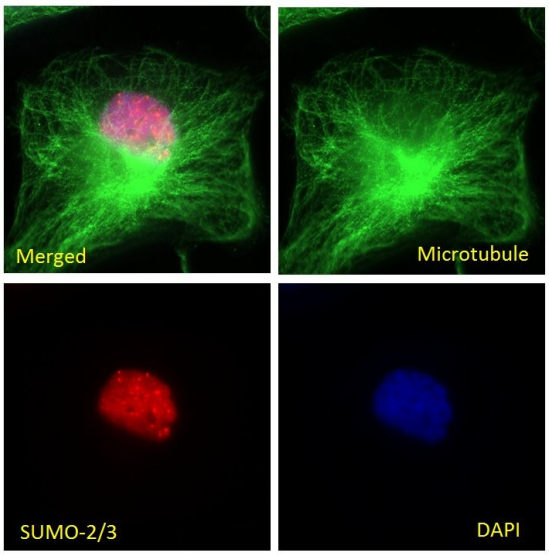



Legend: HeLa cells were stained and visualized by confocal fluorescence microscopy as described in the IF method below. The cells were stained against α/β-tubulin (sheep anti-tubulin Ab, Cat# ATN02, green) and SUMO-2/3 (12F3, red). DNA was stained with DAPI. Mitotic cells in metaphase were imaged with 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 reported[10].

**IF Method for Mitotic Cells**

1. Plate HeLa cells at 3 x $10^5$/ml in glass bottom dish (MatTeck cat# P35G-1.5-14-C) with DMEM media containing 10% FBS.
2. Allow cells to grow for 24-48 h to reach 80% confluency.
3. 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.
4. Wash the cell plate with PBS briefly. Add 3 ml of 4% paraformaldehyde solution and incubate for 10min at room temperature.
5. Wash the cell plate two times with PBS (10 min incubation per wash, no agitation).
6. (Optional) Incubate in 3ml of blocking solution (e.g. PBS containing 3% BSA) for 30 min. Aspirate blocking solution.
7. Apply 1 ml of 12F3 solution (1:500 in PBS) containing other probing reagent (e.g. anti-tubulin antibody and DAPI).
8. Incubate at room temperature for 45 min.
9. Wash the cell plate two times with PBS (10 min incubation per wash, no agitation.
10. 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).
11. Incubate at room temperature for 45 min.
12. Wash the cell plate two times with PBS over 20 min.
13. 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 reported[11].

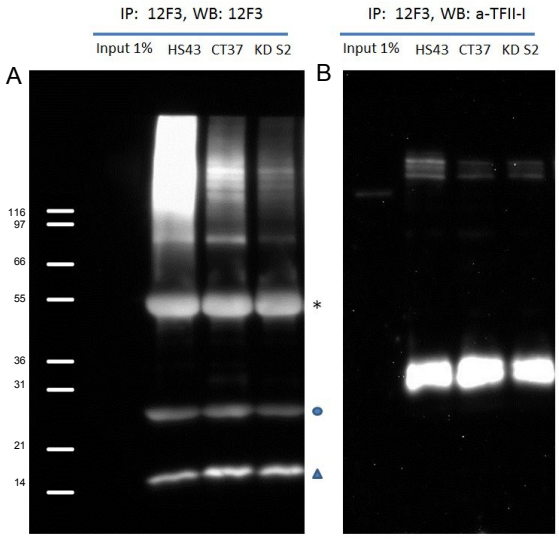
**IF Method for non-mitotic cells**

1. Plate HeLa cells at 3 x $10^5$/ml in glass bottom dish (MatTeck cat# P35G-1.5-14-C) with DMEM media containing 10% FBS.
2. Allow cells to grow for 24-48 h to reach 80% confluence.
3. Fix cells by adding 3ml of 4% paraformaldehyde solution and by incubating for 10min at room temperature.
4. Wash the cell plate with PBS briefly (2 minute incubation, no agitation).
5. Permeabilize cells by incubating in 3ml of 0.5% Triton X-100 solution for 5 min at room temperature.
6. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
7. (Optional) Incubate in 3ml of blocking solution (e.g. PBS containing 3% BSA) for 30 min. Aspirate blocking solution.
8. Apply 1ml of 12F3 solution (1:500 in PBS) containing other probing reagent (e.g. anti-tubulin antibody and DAPI).
9. Incubate at room temperature for 45 min.
10. Wash the cell plate two times with PBS (10 min. incubation per wash, no agitation).
11. 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).
12. Incubate at room temperature for 45 min.
13. Wash the cell plate two times with PBS (10 min incubation per wash, no agitation).
14. 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 S2[12]. 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 µl 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 resuspention with washing buffer (50mM Tris pH7.5, 150mM NaCl, 1% IGEPAL).
4. Resuspend beads in 30 µl 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 µl) after centrifugation (960 x g, 1 min., room temp).
6. Boil the sample with 1 µl of beta mercaptoethanol for 5 min prior to loading on SDS-PAGE for subsequent Western blot analysis.

**References**

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