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Product datasheet for AM26638AF-N

Sumo 1 (SUMO1) Mouse Monoclonal Antibody [Clone ID: 5B12]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5B12
Applications:	IF, WB
Recommended Dilution:	Western blot: 1 µg/ml for chemiluminescence detection system. Immunoflourescence: 5 µg/ml. For details see protocols below.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant full-length GST-SUMO-1
Specificity:	This antibody reacts with SUMO-1 (15~17kDa).
Formulation:	PBS containing 50% glycerol, pH 7.2. Contains no preservatives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliqouts) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	small ubiquitin-like modifier 1
Database Link:	Entrez Gene 7341 Human P63165

View online »

Background: Sumoylation, the covalent attachment of a small ubiquitin-like modifier (SUMO) peptide to lysine residues of targeted substrate, has recently emerged as an important mechanism in transcriptional control. The 15~17 kDa SUMO-1/UBL1/Sentrin is processed by SUMO hydrolase / isopeptidase to generate a free glycine residue that covalently attaches to protein substrates. Unlike ubiquitin, SUMO-1 does not appear to target proteins for degradation but seems to be involved in the modulation of protein-protein interactions. SUMO modification represses the activity of targeted transcriptional activators by altering their subcompartmentalization and binding properties. Sumoylation also recruits histone deacetylases, leading to SUMO-dependent transcriptional repression. Major SUMO-1 substrates include RanGAP1, PML, SP100, p53, Mdm2, c-Jun, topoisomerase I and II, and Iκ B.

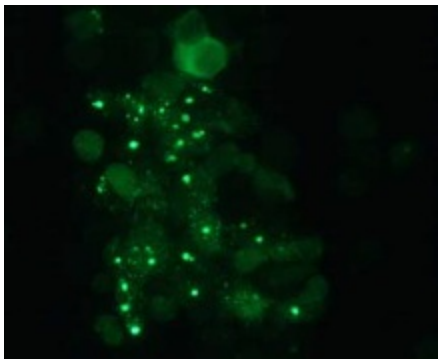
Synonyms: SMT3C, SMT3H3, UBL1, GMP1, SMT3 homolog 3, Sentrin

Note: This product was originally produced by MBL International.

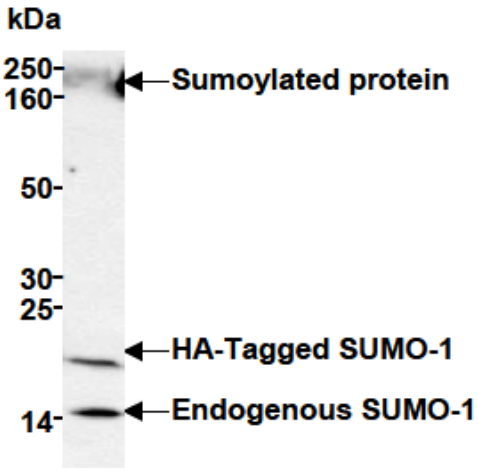
Protocol:
SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% NP-40, with or without 20 mM N-ethylmaleimide [NEM]) containing appropriate protease inhibitors. Incubate it at 4 oC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4 oC and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with an equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 oC.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS (5 minutes x 6 times).
11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
12) Expose to X-ray film in a dark room for 5 minutes. Develop the film as usual. The conditions for exposure and development may vary.

(Positive controls for western blotting ; transfectant, 293T)
Immunofluorescence microscopy
1) Detach the cells (5x10e5 cells) from culture dish by pipetting.
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in cold 4% PFA for 10 minutes at room temperature.
4) Wash the cells 2 times with PBS.
5) Add 30 µL of the anti-SUMO-1 monoclonal antibody (5B12) (5 µg/mL) diluted with PBS containing 0.1% triton-X100. Mix well, and incubate for 30 minutes at room temperature.
6) Add 1 mL of PBS followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 30 µL of 1:100 FITC conjugated anti-mouse IgG diluted with blocking buffer onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
8) Add 1 mL of PBS followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with mounting medium.
10) Drop the cell suspension onto a glass slide, then put a cover slip on it.
(Positive control for immunocytochemistry; transfectant)

Product images:



Immunocytochemical detection of SUMO-1 on 4% PFA fixed HA-tagged SUMO-1 transfected 293T cells with AM26638AF-N.



Western blot analysis of SUMO-1 expression in HA tagged SUMO-1 transfected 293T cells using AM26638AF-N.