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Product datasheet for AM26639AF-N

Sumo 2 (SUMO2) Mouse Monoclonal Antibody [Clone ID: 1E7]

Product data:

Product Type:	Primary Antibodies
Clone Name:	1E7
Recommended Dilution:	Western blot: 2 µg/ml for chemiluminescence detection system. Immunoflourescence: 5 µg/ml. For details see protocols below.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Recombinant full-length human SUMO-2
Specificity:	This antibody reacts with SUMO-2/3 (15 kDa) but not react with SUMO-1 (15~17 kDa) on Western blot.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. 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 2
Database Link:	Entrez Gene 6613 Human P61956

View online »

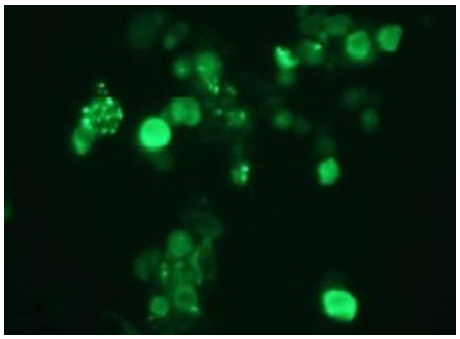
Background: Sumoylation, the covalent attachment of a small ubiquitin-like modifier (SUMO) peptide to lysine residues of targeted substrate, has recently emerged as an important mechanism in transcriptional control. In humans and mice, the SUMO family consists of three members, SUMO-1, -2, and -3. SUMO-2 (SMT3A, Sentrin-3) and SUMO-3 (SMT3B, Sentrin-2) are similar (~95% identical), but less closely related to SUMO-1 (~50% identical). Whereas many proteins modified by SUMO-1 were identified, many as yet unidentified proteins are modified by SUMO-2/3 after exposure of cells to various stress stimuli.

Synonyms: SMT3A, SMT3H2, HSMT3, SMT3 homolog 2, SUMO-3, Sentrin-2, Smt3A

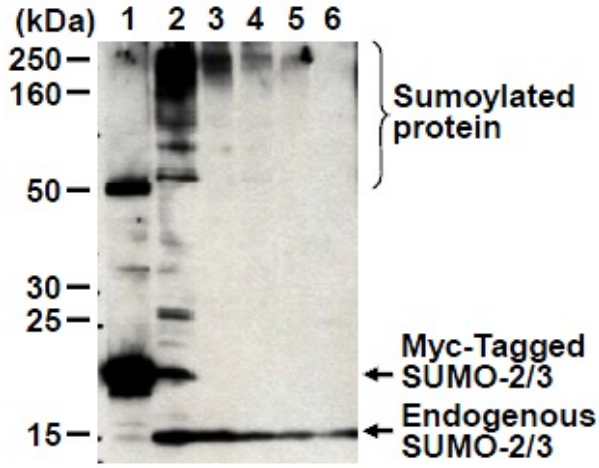
Note: This product was originally produced by MBL International.

Protocol:
SDS-PAGE & Western Blotting
1) Wash the 1x10e7 cells 3 times with PBS and suspend with 1 mL of 1x Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) 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.
4) 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 4oC.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) 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.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 10 minutes. Develop the film as usual. The condition for exposure and development may vary.
(Positive controls for Western blotting; transfectant, 293T, HeLa, NIH/3T3, PC12)
Immunocytochemistry
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% paraformaldehyde for 10 minutes at room temperature.
4) Wash the cells 2 times with PBS.
5) Add 30 µL of the anti-SUMO-2/3 monoclonal antibody (1E7) (5 µg/mL) diluted with PBS containing 0.1% Triton X-100. 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 glass slide then put a cover slip on it.
(Positive control for Immunocytochemistry; transfectant)

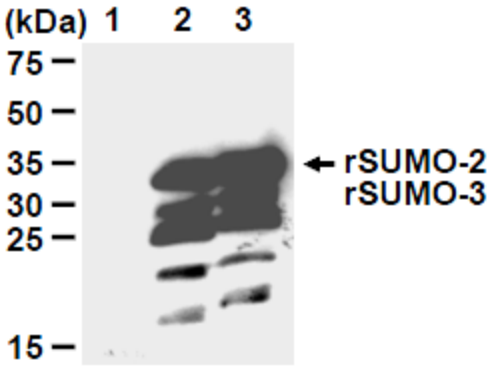
Product images:



Immunoflourescence detection of SUMO2/3 on 4% PFA fixed SUMO2/3 transfected 293T cells with AM26639AF-N



Western blot analysis of SUMO-2/3
Lane 1, 2: Myc-tagged SUMO-2/3 transfected 293T
Lane 3: 293T
Lane 4: HeLa
Lane 5: NIH/3T3
Lane 6: PC12
Immunoblot
Lane 1: with anti-Myc-tag antibody
Lane2-6: with AM26639AF-N



Western blot analysis of SUMO-1, SUMO-2 and SUMO-3

Lane 1: recombinant SUMO-1
Lane 2: recombinant SUMO-2
Lane 3: recombinant SUMO-3
Immunoblotted with AM26639AF-N