**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

Anti-SUMO-1

Code No.	Clone	Subclass	Quantity	Concentration
M113-3	5B12	Mouse IgG1	100 µg	1 mg/mL

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 I-κB.

SOURCE: This antibody was purified from hybridoma (clone 5B12) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with recombinant full-length human SUMO-1.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with SUMO-1 on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not recommended
Immunocytochemistry; 5 µg/mL
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	293T, HeLa, Raji, HL-60	NIH/3T3, WR19L	PC12
Reactivity on WB	+	+	+

REFERENCES:

1) Tiefenbach, J., *et al*., *Mol. Biol. Cell* **17**, 1643-1651 (2006)
2) Kawabe, Y., *et al*., *J. Biol. Chem.* **275**, 20963-20966 (2000)

RELATED PRODUCTS:

M114-3	Anti-SUMO-2/3 (1E7)
D058-3	Anti-Multi Ubiquitin (FK2)
D071-3	Anti-Multi Ubiquitin (FK1)
MK-11-3	Anti-Ubiquitin (1B3)
MK-12-3	Anti-Ubiquitin (2C5)
PM023	Anti-NEDD8 (polyclonal)

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% NP-40, with or without 20 mM NEM) containing appropriate protease inhibitors. Incubate it at 4°C 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°C 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 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/cm^2 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°C.

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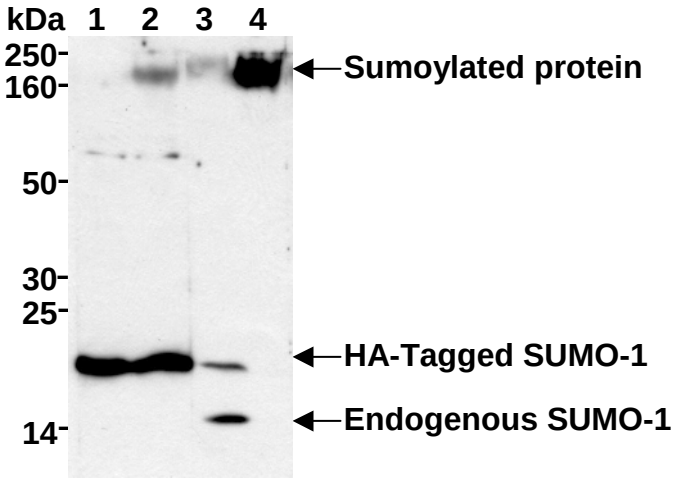
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) 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.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, Raji, HL-60, NIH/3T3, WR19L, PC12)

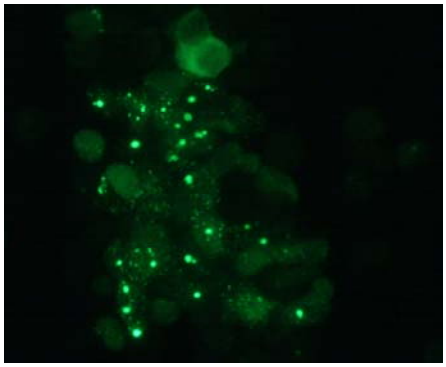


Western blot analysis of SUMO-1 [NEM(-) 1 and 3, NEM(+) 2 and 4] expression in HA tagged SUMO-1 transfected 293T cells using M107-3 (1 and 2) and M113-3 (3 and 4).

Immunocytochemistry

1) Detach the cells ($5x10^5$ 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-1 monoclonal antibody (5B12) (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 (MBL; code no. IM-0819) 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)



Immunocytochemical detection of SUMO-1 on 4% PFA fixed HA-tagged SUMO-1 transfected 293T cells with M113-3.