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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-SUMO-2/3 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M114-3	1E7	Mouse IgG2b	100 µL	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. 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.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with the recombinant full-length human SUMO-3.

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-2/3 (15 kDa) but not react with SUMO-1 (15~17 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 2 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not recommended
Immunocytochemistry; 5 µg/mL
Flow cytometry; Not tested
Chromatin Immunoprecipitation; Not tested*
Other; Not tested*

*It is reported that this antibody can be used in Chromatin Immunoprecipitation[6] and Proximity ligation assay[3].

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

INTENDED USE:

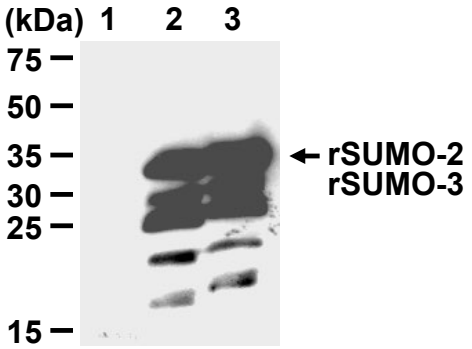
For Research Use Only. Not for use in diagnostic procedures.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Transfectant, 293T, HeLa	NIH/3T3	PC12
Reactivity on WB	+	+	+

REFERENCES:

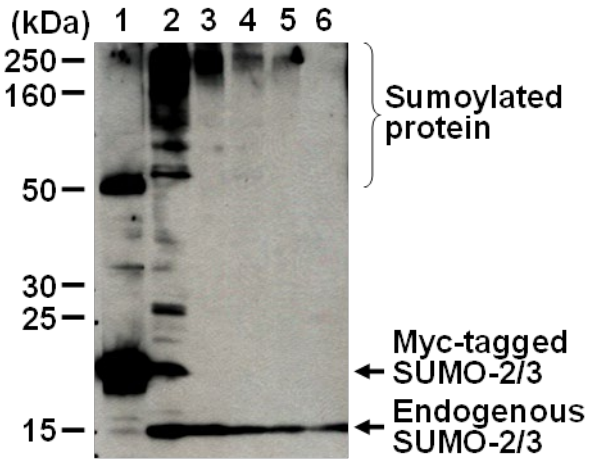
1) Eifler, K., *et al*., *Nat. Commun*. **9**, 1119 (2018) [WB]
2) Ambaye, N., *et al., Biochemistry* **57**, 1807-1813 (2018) [WB]
3) Gilistro, E., *et al., J. Cell Sci*. **130**, 2564-2578 (2017) [PLA]
4) Du, L., *et al.*, *Nat. Commun*. **7**, 12326 (2016) [WB]
5) Lecona, E., *et al.*, *Nat. Struct. Mol. Biol.* **23**, 270-277 (2016) [WB, IC]
6) Niskanen, E. A., *et al.*, *Genome Biol.* **16**, 153 (2015) [WB, ChIP]
7) Yan, Y., *et al.*, *Nat. Commun*. **6**, 8979 (2015) [WB]
8) Sutinen, P., *et al., Mol. Endocrinol.* **28**, 1719-1728 (2014) [WB]
9) Paakinaho, V., *et al., Mol. Cell Biol*. **34**, 202-213 (2014) [WB]
10) Berndt, A., *et al., Biochem. J*. **456**, 385-395 (2013) [WB]
11) Guo, C., *et al., EMBO J.* **32**, 1514-1528 (2013) [WB]
12) Psakhye, I. and Jentsch, S., *Cell* **151**, 807-820 (2012) [WB]
13) Fanis, P., *et al., Mol. Cell Proteomics* **11**, 1263-1273 (2012) [WB]
14) Finkbeiner, E., *et al., EMBO J*. **30**, 1067-1078 (2011) [WB]
15) Fernández-Miranda, G, *et al.*, *J. Cell Sci.* **123**, 2823-2833 (2010) [WB]
16) Du, J. X., *et al.*, *J. Biol. Chem.* **285**, 28298-28308 (2010) [WB]
17) Klein, U. R., *et al.*, *Mol. Biol. Cell* **20**, 410-418 (2009) [WB]
18) Müller, S., *et al*., *Nat. Rev. Mol. Cell Biol.* **2**, 202-210 (2001)
19) Saitoh, H., *et al*., *J. Biol. Chem.* **275**, 6252-6258 (2000)



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

Lane 1: recombinant SUMO-1
Lane 2: recombinant SUMO-2
Lane 3: recombinant SUMO-3

This data indicates that M114-3 reacts with SUMO-2 and -3 specifically but not SUMO-1.



Western blotting analysis of SUMO-2/3

Sample

Lane 1, 2: Myc-tagged SUMO-2/3 transfected 293T
Lane 3: 293T
Lane 4: HeLa
Lane 5: NIH/3T3
Lane 6: PC12

Immunoblotting

Lane 1: Anti-Myc-tag mAb (MBL, code no. M047-3)
Lane 2-6: Anti-SUMO-2/3 mAb (MBL, code no. M114-3)

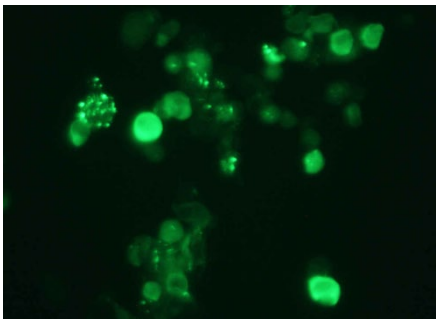
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash the 1x10^7 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/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% Methanol). 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 4°C.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
7) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) 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).
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 and PC12)



Immunocytochemical detection of SUMO-2/3 on 4% PFA fixed SUMO-2/3 transfected 293T cells with M114-3

Immunocytochemistry

1) Detach the cells (5 x 10^5 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 twice with PBS.
5) Add 30 µL of the primary antibody diluted with PBS containing 0.1% Triton X-100 as suggested in the **APPLICATIONS**. 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 FITC-conjugated anti-mouse IgG antibody 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)

RELATED PRODUCTS:

Please visit our website at https://ruo.mbl.co.jp/.