

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

Anti-SMN1 pAb

CODE No.	RN077PW
CLONALITY	Polyclonal
ISOTYPE	Rabbit Ig, affinity purified
QUANTITY	100 µL
SOURCE	Purified Ig from rabbit serum
FORMULATION	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.

APPLICATIONS-CONFIRMED

<u>Western blotting</u>	1:1,000
<u>Immunoprecipitation</u>	5 µL/500 µL of cell extract from 1 x 10 ⁷ cells

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	293T, HeLa, Jurkat, K562	NIH/3T3, WR19L	Rat1	CHO
Reactivity	+	-	-	-

Entrez Gene ID 6606 (Human)

For more information, please visit our website at <https://ruo.mbl.co.jp/>.

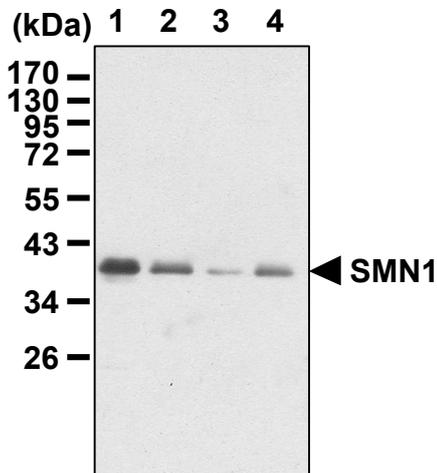
The descriptions of the following protocols are examples.

Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 seconds).
- 2) Boil the samples for 3 minutes and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) 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 4°C.
- 5) Wash the membrane with PBS-T (5 minutes x 3 times).
- 6) 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 concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3 times).
- 8) Incubate the membrane with the 1:5,000 anti-rabbit IgG-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 9) Wash the membrane with PBS-T (10 minutes x 3 times).
- 10) 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.
- 11) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, Jurkat and K562)



Western blot analysis of SMN1

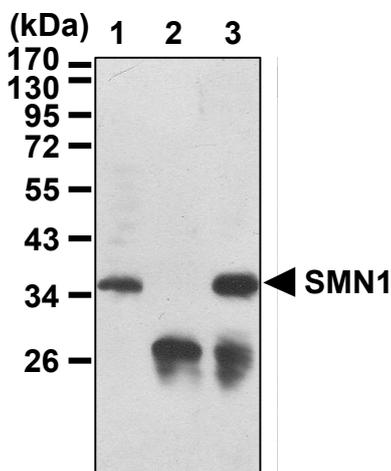
Lane 1: 293T
Lane 2: HeLa
Lane 3: Jurkat
Lane 4: K562

Immunoblotted with RN077PW

Immunoprecipitation

- 1) Wash 2×10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (150 mM NaCl, 20 mM Tris-HCl, pH 8.0, 0.1% NP-40, 10 mM EDTA) containing appropriate protease inhibitors. Vortex for 10 seconds, then leave on ice for 10 minutes.
- 2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add 40 μ L of 50% protein A agarose beads slurry resuspended in Lysis Buffer into the supernatant. Incubate it at 4°C with rotating for 1 hour.
- 4) Centrifuge the tube at 2,000 x g for 2 minutes at 4°C and transfer the supernatant to another tube (precleared sample).
- 5) Mix 20 μ L of 50% protein A agarose beads slurry resuspended in PBS with normal rabbit IgG (RIP-Assay Kit) or anti-SMN1 pAb at the amount of suggested in the **APPLICATIONS**, then add 1 mL of Lysis Buffer into each tube. Incubate with gentle agitation for 1 hour at 4°C.
- 6) Wash the beads once with ice-cold Lysis Buffer (centrifuge the tube at 2,000 x g for 1 minute). Carefully discard the supernatant using a pipettor without disturbing the beads.
- 7) Add 500 μ L of cell lysate (precleared sample from step 4), then incubate with gentle agitation for 3 hours at 4°C.
- 8) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 minute).
- 9) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 3 minutes, and centrifuge for 5 minutes. Use 20 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 10) 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.
- 11) 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.
- 12) Wash the membrane with PBS-T (10 minutes x 3 times).
- 13) 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 concentration of antibody will depend on the conditions.)
- 14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3 times).
- 15) Incubate the membrane with the 1:1,000 Rabbit TrueBlot[®] anti-Rabbit IgG-HRP (eBioscience; code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 16) Wash the membrane with PBS-T (10 minutes x 3 times).
- 17) 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.
- 18) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



Immunoprecipitation of SMN1 from 293T

Lane 1: Input
Lane 2: IP with normal rabbit IgG
Lane 3: IP with RN077PW
Immunoblotted with RN077PW