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Not for use in diagnostic procedures.

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RiboCluster Profiler™

RBP Antibody

Anti-SRSF9 (SRp30c) pAb

CODE No. RN081PW

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

Western blotting 1:1,000
Immunoprecipitation 5 µL/500 µL of nuclear extract from 5 x 10^6 cells

APPLICATIONS-REPORTED

Immunocytochemistry Reference 1) and 3)
Crosslinking immunoprecipitation (CLIP) Reference 2)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Jurkat, A431	NIH/3T3	Rat1	CHO
Reactivity	+	+	+	+

Entrez Gene ID 8683 (Human), 108014 (Mouse), 288701 (Rat), 100763712 (Hamster)

REFERENCES

1) Markmiller, S., *et al., Cell* **172**, 590-604.e13 (2018) [IC]
2) Van Nostrand, E. L., *et al., Nat. Methods* **13**, 508-514 (2016) [CLIP]
3) Sundararaman, B., *et al., Mol. Cell* **61**, 903-913 (2016) [IC]

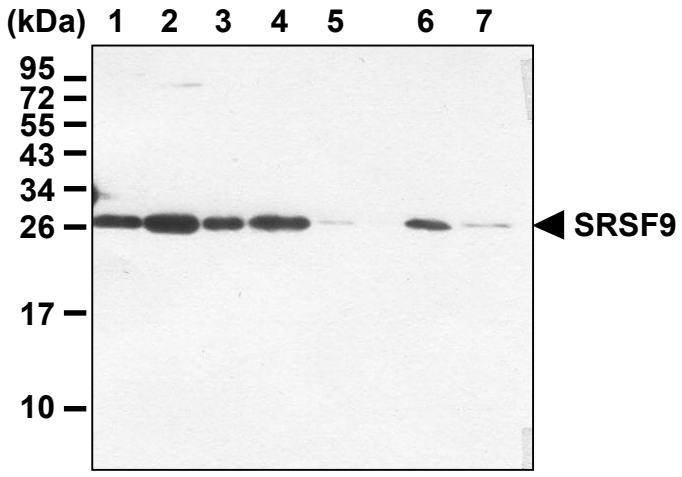
For more information, please visit our website at https://ruo.mbl.co.jp/.

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

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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) overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (10 min. x 3 times).
8) Incubate the membrane with 1:5,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (10 min. x 3 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. 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 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, A431, Jurkat, NIH/3T3, Rat1 and CHO)



Western blot analysis of SRSF9 (SRp30c)

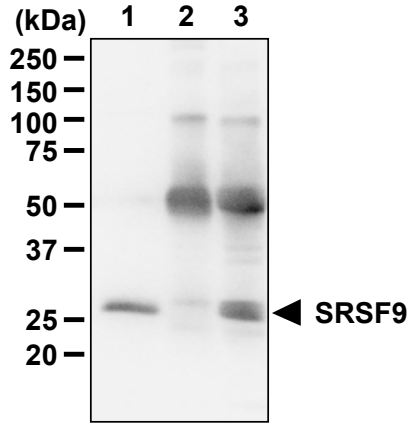
Lane 1: HeLa
Lane 2: 293T
Lane 3: A431
Lane 4: Jurkat
Lane 5: NIH/3T3
Lane 6: Rat1
Lane 7: CHO

Immunoblotted with Anti-SRSF9 (SRp30c) pAb (RN081PW)

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold IP buffer [150 mM NaCl, 20 mM Tris-HCl (pH 8.0), 0.1% NP-40, 10 mM EDTA] containing appropriate protease inhibitors and 1.5 mM DTT. Vortex thoroughly, then incubate it on ice for 10 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and discard the supernatant.
3) Wash the pellet 3 times with PBS and resuspend them with 500 µL RIPA buffer, then sonicate briefly.
4) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
5) Add 500 µL of ice-cold IP buffer into the supernatant. Mix by pipetting up and down.
6) Add 40 µL of 50% protein G agarose beads slurry resuspended in IP buffer into the sample (prepared from step 5). Incubate it at 4°C with rotating for 1 hour.
7) Centrifuge the tube at 2,000 x g for 2 minutes at 4°C and transfer the supernatant to another tube (precleared sample).
8) Mix 20 µL of 50% protein G agarose beads slurry resuspended in PBS with Normal Rabbit IgG (MBL; code no. PM035) or Anti-SRSF9 (SRp30c) pAb (MBL; code no. RN081PW) at the amount of suggested in the **APPLICATIONS,** then add 1 mL of IP buffer into each tube. Incubate with gentle agitation for 1 hr. at 4°C.
9) Wash the beads once with 500 µL of ice-cold IP buffer (centrifuge the tube at 2,000 x g for 1 min.). Carefully discard the supernatant using a pipette or without disturbing the beads.
10) Add 500 µL of nuclear extract (the sample from step 7), then incubate with gentle agitation for 3 hr. at 4°C.
11) Wash the beads 4 times with IP buffer (centrifuge the tube at 2,000 x g for 1 min.).
12) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min., and centrifuge for 5 min. Use 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (5-20% acrylamide, gradient) for electrophoresis.
13) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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.
14) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature, or overnight at 4°C.
15) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
16) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
17) Wash the membrane with PBS-T (10 min. x 3 times).
18) Incubate the membrane with 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 hr. at room temperature.
19) Wash the membrane with PBS-T (10 min. x 3 times).
20) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
21) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T nuclear extract)



Immunoprecipitation of SRSF9 (SRp30c)

Sample: 293T nuclear extract
Lane 1: Input
Lane 2: IP with Normal Rabbit IgG (PM035)
Lane 3: IP with Anti-SRSF9 (SRp30c) pAb (RN081PW)

Immunoblotted with RN081PW