

RiboCluster Profiler™

RBP Antibody

Anti-SRSF5 (SRP40) pAb

CODE No.	RN082PW
CLONALITY	Polyclonal
ISOTYPE	Rabbit Ig, affinity purified
QUANTITY	100 µL, 1 mg/mL
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 for chemiluminescence detection system
<u>Immunoprecipitation</u>	5 µL/500 µL of nuclear extract from 1 x 10 ⁷ cells

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	HeLa, HepG2, Jurkat, 293T	NIH/3T3, WR19L	Rat1	CHO
Reactivity	+	+	+	+

Entrez Gene ID 6430 (Human), 20384 (Mouse), 29667 (Rat), 100757287 (Hamster)

For more information, please visit our web site <https://ruo.mbl.co.jp/je/rip-assay/>

RELATED PRODUCTS

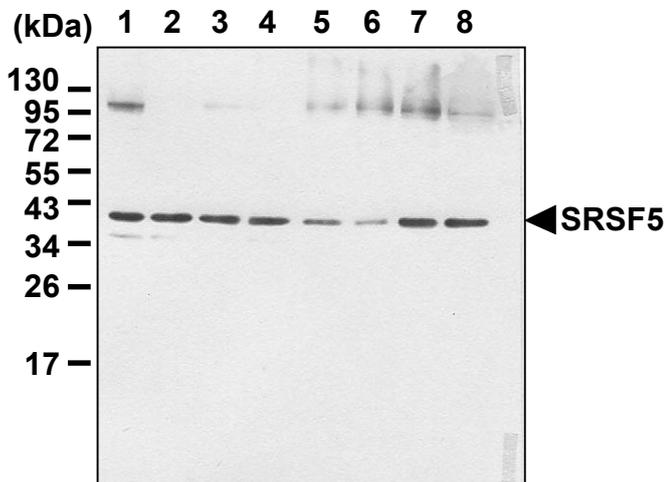
Please visit our web site <https://ruo.mbl.co.jp/>

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 sec.).
- 2) Boil the samples for 3 min. 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 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 of 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, HepG2, Jurkat, 293T, NIH/3T3, WR19L, Rat1 and CHO)



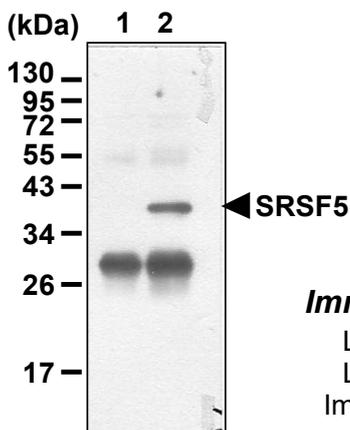
Western blot analysis of SRSF5 (SRP40)

Lane 1: HeLa
Lane 2: HepG2
Lane 3: Jurkat
Lane 4: 293T
Lane 5: NIH/3T3
Lane 6: WR19L
Lane 7: Rat1
Lane 8: CHO
Immunoblotted with RN082PW

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 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 μ L 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 fresh tube.
- 5) Add 500 μ L of ice-cold Lysis buffer into the supernatant. Mix by pipetting up and down.
- 6) Add 40 μ L of 50% protein G agarose beads slurry resuspended in Lysis Buffer into the sample (prepared from step 5). Incubate it at 4°C with rotating for 1 hr.
- 7) Centrifuge the tube at 2,000 x g for 2 min. at 4°C and transfer the supernatant to another tube (precleared sample).
- 8) Mix 20 μ L of 50% protein G agarose beads slurry resuspended in PBS with Normal Rabbit IgG (RIP-Assay Kit) or Anti-SRSF5 (SRP40) pAb (RN082PW) at the amount of suggested in the **APPLICATIONS**, then add 1 mL of Lysis Buffer into each tube. Incubate with gentle agitation for 1 hr. at 4°C.
- 9) Wash the beads once with 500 μ L of ice-cold Lysis 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 μ L 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 Wash Buffer (centrifuge the tube at 2,000 x g for 1 min.).
- 12) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 3 min., and centrifuge for 5 min. Use 20 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 13) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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 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 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; Jurkat nuclear extract)



Immunoprecipitation of SRSF5 (SRP40) from Jurkat nuclear extract

Lane 1: IP with normal rabbit IgG

Lane 2: IP with RN082PW

Immunoblotted with RN082PW