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

Anti-FUSIP1 (SRSF10) pAb

CODE No. RN064PW

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

Western blotting 1:1,000
Immunoprecipitation 5 µL/500 µL cell extract from 1 x 10^7 cells

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Jurkat, HepG2	NIH/3T3	Not tested	CHO
Reactivity	+	+		+

Entrez Gene ID 10772 (Human), 14105 (Mouse)

RELATED PRODUCTS

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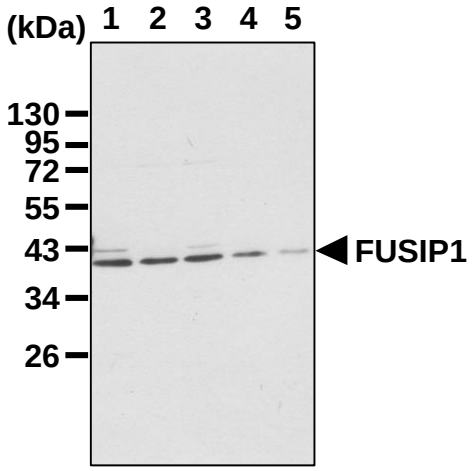
SDS-PAGE & Western blotting

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 seconds).
2) Boil the samples for 3 minutes and centrifuge. Load 10 μL 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/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) Wash the membrane with PBS-T (5 minutes x 3).
6) 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.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3).
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).
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 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, HepG2, Jurkat and NIH/3T3)

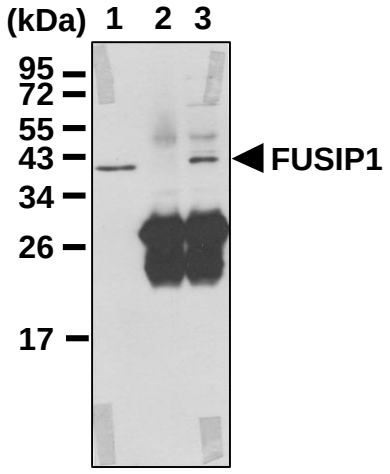


Western blotting analysis of FUSIP1
Lane 1: 293T
Lane 2: HeLa
Lane 3: HepG2
Lane 4: Jurkat
Lane 5: NIH/3T3
Immunoblotted with RN064PW

Immunoprecipitation

1) Wash 2 x 10^7 cells twice 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 µL 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 µL of 50% protein A agarose beads slurry resuspended in PBS with normal rabbit IgG (RIP-Assay Kit, MBL, code no.RN1001) or anti-FUSIP1 pAb at the amount 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 µL of cell lysate (precleared sample from step 4), then incubate with gentle agitation for 3 hour at 4°C.
8) Wash the beads 4 times with ice-cold Lysis buffer (centrifuge the tube at 2,000 x g for 1 minute).
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 minutes, and centrifuge for 5 minutes. Use 20 µL 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/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.
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 (5 minutes x 3).
13) Incubate the membrane with primary antibody diluted 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.)
14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3).
15) Incubate the membrane with the 1:1,000 Rabbit TrueBlot® anti-Rabbit IgG-HRP (Rockland Immunochemicals, 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).
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 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



Immunoprecipitation of FUSIP1 from 293T
Lane 1: Input
Lane 2: IP with normal rabbit IgG
Lane 3: IP with RN064PW
Immunoblotted with RN064PW