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Not for use in diagnostic procedures.

MBL
A JSR Life Sciences Company

RiboCluster Profiler™

RBP Antibody

Anti-ALKBH5 pAb

CODE No. RN122PW

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

Western blotting 1:1,000 for chemiluminescence detection system
Immunoprecipitation 5 μL/500 μL of cell extract from 5 x 10^6 cells/sample

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse*	Rat	Hamster
Cells	HeLa, HEK293T, Jurkat, K562	WR19L	Rat1	CHO
Reactivity	+	+	-	-

*This antibody shows weak reactivity with NIH/3T3 cells.

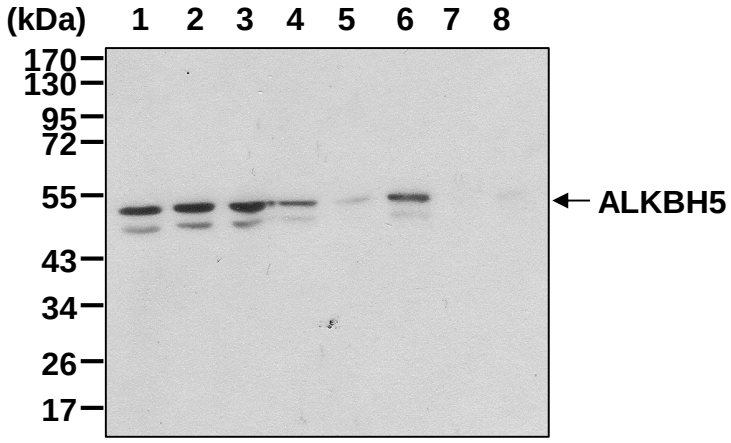
Entrez Gene ID 54890 (Human), 268420 (Mouse)

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

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 (10% 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 manufacturer'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 the 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 settings. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, HEK293T, Jurkat, K562 and WR19L)



Western blot analysis of ALKBH5

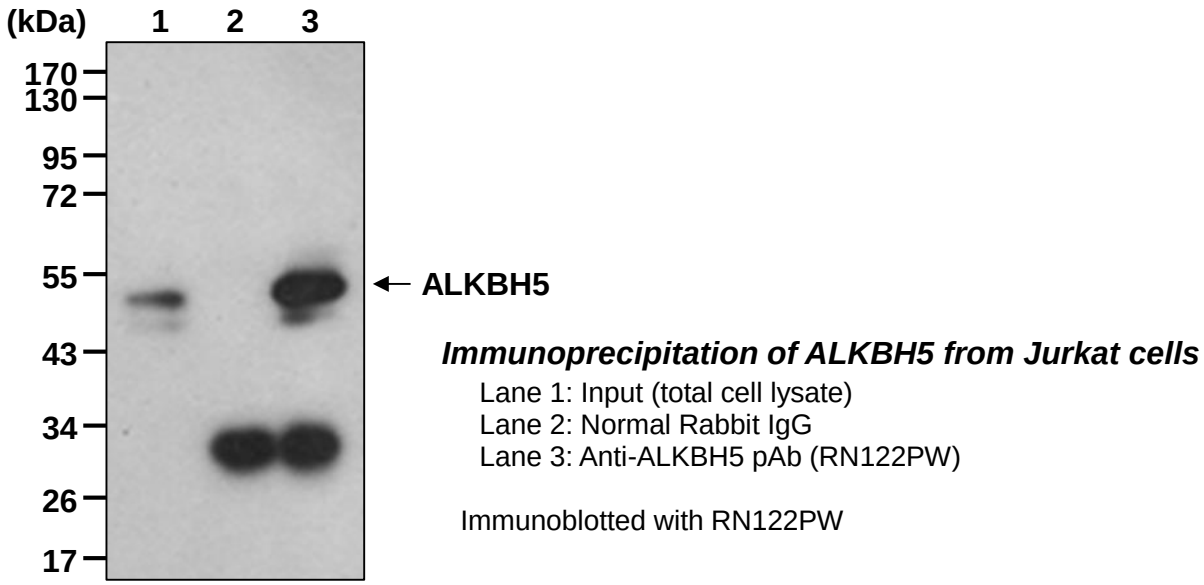
Lane 1: HeLa
Lane 2: HEK293T
Lane 3: Jurkat
Lane 4: K562
Lane 5: NIH/3T3
Lane 6: WR19L
Lane 7: Rat1
Lane 8: CHO

Immunoblotted with Anti-ALKBH5 pAb (RN122PW)

Immunoprecipitation

1) Wash 1 x 10^7 cells 4 times with PBS and resuspend them with 1 mL of ice-cold Lysis Buffer (+) (MBL; code no. RN1001) containing appropriate protease inhibitors and DTT. Vortex thoroughly, then incubate on ice for 10 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Add 20 µL of 50% protein G agarose beads slurry resuspended in ice-cold Wash Buffer (+) (MBL; code no. RN1001) containing DTT at the appropriate concentration into the supernatant. Incubate it at 4°C with rotating for 1 hr.
4) Centrifuge the tube at 2,000 x g for 1 min. at 4°C and transfer the supernatant to another tube (precleared sample).
5) Mix 20 µL of 50% protein G agarose beads slurry resuspended in 1 mL of ice-cold Wash Buffer (+) with Normal Rabbit IgG (RIP-Assay Kit) or Anti-ALKBH5 pAb (MBL; code no. RN122PW) as suggested in the **APPLICATIONS**. Incubate at 4°C with rotating for 1 hr.
6) Wash the beads 1 time with ice-cold Lysis Buffer (+). Carefully discard the supernatant.
7) Add 500 µL of the precleared sample (prepared in step 4)) to the tube containing antibody conjugated beads, then incubate with gentle agitation for 2 hr. at 4°C.
8) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
9) Resuspend the beads with 1 mL of ice-cold Wash Buffer (+).
10) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
11) Repeat steps 8)-10) 3 times.
12) Resuspend the bead pellet in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
13) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
14) 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 manufacturer's manual for precise transfer procedure.
15) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
16) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
17) 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.)
18) Wash the membrane with PBS-T (10 min. x 3 times).
19) Incubate the membrane with 1:1,000 of 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.
20) Wash the membrane with PBS-T (10 min. x 3 times).
21) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min.
22) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
23) 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)



Immunoprecipitation of ALKBH5 from Jurkat cells

Lane 1: Input (total cell lysate)
Lane 2: Normal Rabbit IgG
Lane 3: Anti-ALKBH5 pAb (RN122PW)

Immunoblotted with RN122PW

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