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Anti-NR1D1 (Rev-erbα) pAb

CODE No. PM092

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 1:200-1:1,000 for chemiluminescence detection system
Immunoprecipitation 2 µL/sample

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	U2OS	Liver nuclear extract, NIH3T3-3-4, transfectant	Rat-1	Not tested
Reactivity	+	+	+	

Entrez Gene ID 9572 (Human), 217166 (Mouse), 252917 (Rat)

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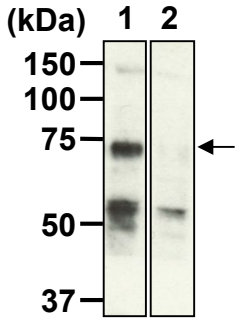
The descriptions of the following protocols are examples. Each user should determine the appropriate condition

SDS-PAGE & Western blotting

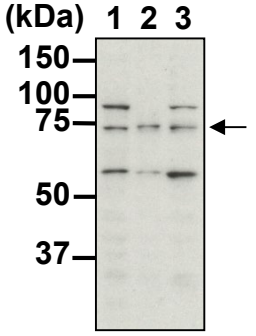
1) Prepare the samples described as below:
   [Tissue] Mix 5 μL of mouse liver nuclear extract with 5 μL of Laemmli's sample buffer.
   [Cell line] Wash 1 x 10^7 cells 3 times with PBS and suspends them in 500 μL of Laemmli's sample buffer.
   [Transfectant] Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 5 min. and centrifuge. Load 10 μL of the samples from tissue and cell line or 1 μL of sample from transfectant 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 10% 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 **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3 times).
8) Incubate the membrane with 1:10,000 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 (5 min. x 3 times).
10) Wipe excess buffer on the membrane, and 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; Mouse liver nuclear extract, NIH3T3-3-4, Rat-1, U2OS and transfectant)



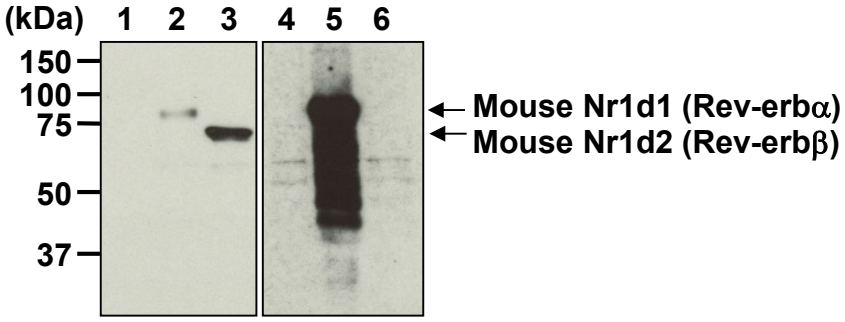
Western blot analysis of mouse Nr1d1 (Rev-erbα) from liver nuclear extract
Lane 1: ZT10 (zeitgeber time; 10 h)
Lane 2: ZT22 (zeitgeber time; 22 h)

Immunoblotted with Anti-NR1D1 (Rev-erbα) pAb (PM092), 1:1,000



Western blot analysis of NR1D1 (Rev-erbα)
Lane 1: NIH3T3-3-4
Lane 2: Rat-1
Lane 3: U2OS

Immunoblotted with Anti-NR1D1 (Rev-erbα) pAb (PM092), 1:200



Western blot analysis of mouse Nr1d1 (Rev-erbα) from HEK293T transfectant

Lane 1, 4: HEK293T
Lane 2, 5: Myc-tagged mouse Nr1d1/HEK293T
Lane 3, 6: Myc-tagged mouse Nr1d2/HEK293T

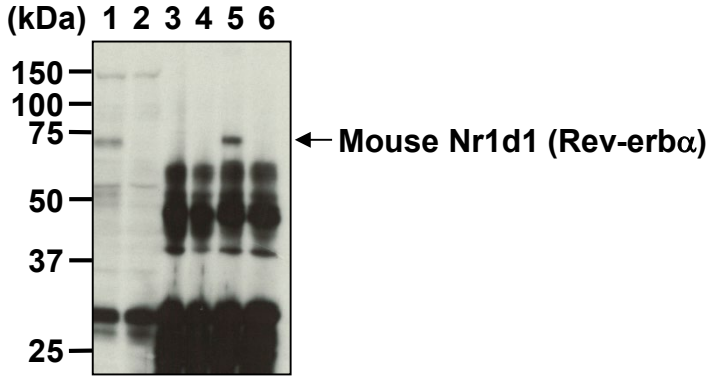
<Immunoblot>
Lane 1-3: 1st antibody; Anti-Myc-tag mAb (M047-3), 1 μg/mL
2nd antibody; Anti-IgG (Mouse) pAb-HRP (330)
Lane 4-6: 1st antibody: Anti-NR1D1 (Rev-erbα) pAb (PM092), 1:1,000
2nd antibody: Anti-IgG (Rabbit) pAb-HRP (458)

Exposure time: 15 min.

Immunoprecipitation

1) Mix 50 µL of mouse liver nuclear extract to 100 µL of IP buffer [20 mM HEPES-NaOH (pH7.8), 5.5 mM NaCl, 1 mM EDTA, 6.5% glycerol, 1.5% Triton X-100, 1 mM DTT, 50 mM NaF, 1 mM Na_3VO_4] containing appropriate protease inhibitors.
2) Add 40 µL of 50% protein A agarose beads slurry resuspended in IP buffer. Incubate it at 4°C with rotating for 30 min.
3) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube (precleared sample).
4) Add primary antibody as suggested in the **APPLICATIONS** to the 150 µL of precleared sample (prepared sample from step 3)). Incubate with gentle agitation for 1 hr. at 4°C.
5) Mix 30 µL of 50% protein A agarose beads slurry into the tube. Incubate with gentle agitation for 1 hr. at 4°C.
6) Wash the beads 4 times with 1 mL of IP buffer.
7) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
8) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
9) 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.
10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
12) Incubate the membrane with 1:1,000 of Anti-NR1D1 (Rev-erbα) pAb (PM092) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
13) Wash the membrane with PBS-T (5 min. x 3 times).
14) Incubate the membrane with the 1:10,000 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.
15) Wash the membrane with PBS-T (5 min. x 3 times).
16) Wipe excess buffer on the membrane, and 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.
17) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Mouse liver nuclear extract)



Immunoprecipitation of mouse Nr1d1 (Rev-erbα) from liver nuclear extract

<Sample>
Lane 1, 3, 5: ZT10 (zeitgeber time; 10 h)
Lane 2, 4, 6: ZT22 (zeitgeber time; 22 h)

Lane 1, 2: Input
Lane 3, 4: Normal Rabbit IgG (PM035)
Lane 5, 6: Anti-NR1D1 (Rev-erbα) pAb (PM092)

Immunoblotted with PM092