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

Anti-NR1D2 (Rev-erbβ) pAb

CODE No. PM093

CLONALITY Polyclonal
ISOTYPE Guinea pig Ig, affinity purified
QUANTITY 100 μL

SOURCE Purified Ig from guinea pig serum
FORMURATION 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	1:200-1:500 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	Not tested	Not tested
Reactivity	+	+		

Entrez Gene ID 9975 (Human), 353187 (Mouse)

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

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
e-mail support@mbl.co.jp, TEL 052-238-1904

RELATED PRODUCTS

Antibodies

PM093	Anti- NR1D2 (Rev-erbβ) pAb
PM092	Anti-NR1D1 (Rev-erbα) pAb
PM091	Anti-Per1 (Mouse) pAb
PM083	Anti-Per2 (Mouse) pAb
PM082	Anti-Cry2 (Mouse) pAb
PM081	Anti-Cry1 (Mouse) pAb
D333-3	Anti-CLOCK (Mouse) mAb (CLSP3)
D334-3	Anti-CLOCK (Mouse) mAb (CLNT1)
D335-3	Anti-BMAL1 (Mouse) mAb (B1BH2)
D349-3	Anti-CLOCK (Mouse) mAb (CLSP4)
PM079	Anti-DBP (Mouse) pAb
CY-P1016	Anti-SIRT1 pAb
RN032P	Anti-CIRBP pAb
RN013MW	Anti-Nono (P54NRB) mAb (C5)
RN014MW	Anti-SFPQ (PSF) mAb (C23)
RN015MW	Anti-PSPC1 (PSP1) mAb (1L4)
RN092PW	Anti-NONO (P54NRB) pAb
RN106PW	Anti-SFPQ (PSF) pAb
PM075	Anti-GNAT2 (Zebrafish) pAb
PM067	Normal Guinea Pig IgG

Kits

CY-1151	CycLex® SIRT1/Sir2 Deacetylase Fluorometric Assay Kit
CY-1152	CycLex® SIRT2 Deacetylase Fluorometric Assay Kit
CY-1173	CycLex® CaM-kinase II Assay Kit
CY-8102	CircuLex Mouse CIRP ELISA Kit
CY-8103	CircuLex Human CIRP ELISA Kit

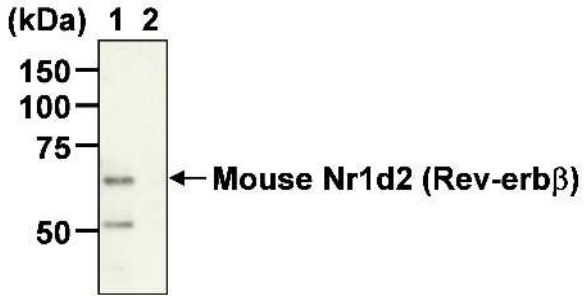
Recombinant proteins (Human, Active)

CY-E1151	NAD^+-Dependent Deacetylase SIRT1
CY-E1152	NAD^+-Dependent Deacetylase SIRT2
CY-E1173	CaM-kinase II Positive Control

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 1:20,000 of Rabbit anti-Guinea Pig IgG (H+L) Secondary Antibody, HRP conjugate (Life Technologies; code no. 61-4620) 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.

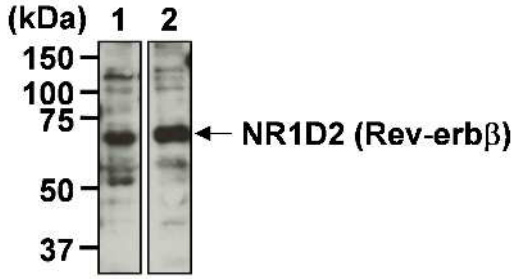
(Positive controls for Western blotting; Mouse liver nuclear extract, NIH3T3-3-4, U2OS and transfectant)



Western blot analysis of mouse Nr1d2 (Rev-erbβ) from liver nuclear extract

Lane 1: ZT10 (zeitgeber time; 10 h)
Lane 2: ZT22 (zeitgeber time; 22 h)

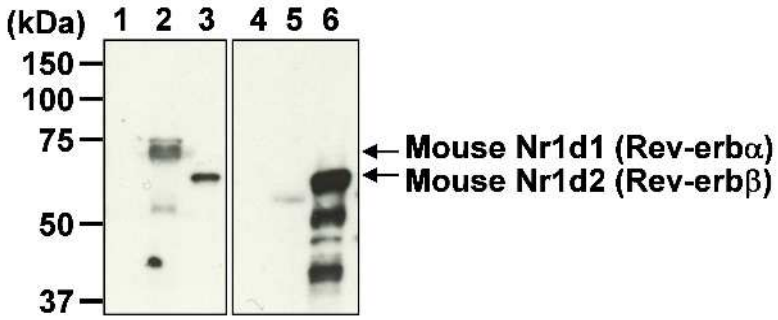
Immunoblotted with Anti-NR1D2 (Rev-erbβ) pAb (PM093), 1:500



Western blot analysis of NR1D2 (Rev-erbβ)

Lane 1: NIH3T3-3-4
Lane 2: U2OS

Immunoblotted with Anti-NR1D2 (Rev-erbβ) pAb (PM093), 1:200



Western blot analysis of mouse Nr1d2 (Rev-erbβ) from HEK293T transfectant

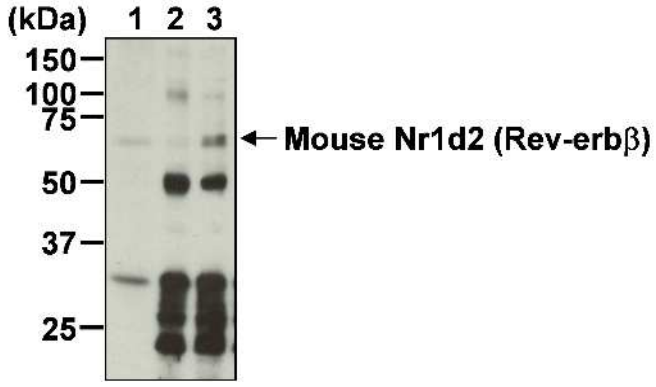
Lane 1, 4: HEK293T
Lane 2, 5: Myc-tagged mouse Nr1d1/HEK293T
Lane 3, 6: Myc-tagged mouse Nr1d2/HEK293T

<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-NR1D2 (Rev-erbβ) pAb (PM093), 1:500
2nd antibody: Rabbit anti-Guinea Pig IgG (H+L) Secondary Antibody, HRP conjugate
(Life Technologies; code no. 61-4620)

Immunoprecipitation

1) Wash 1 x 10^7 cells 3 times with PBS and add 1 mL of IP buffer [20 mM HEPES-NaOH (pH7.8), 137 mM NaCl, 1 mM EDTA, 5% glycerol, 1% Triton X-100, 50 mM NaF] containing appropriate protease inhibitors. Sonicate briefly (up to 10 seconds), then incubate it at 4°C for 30 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 100 µL of 50% protein A agarose beads slurry resuspended in PBS. Incubate it at 4°C with rotating for 30 min.
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) Add primary antibody as suggested in the **APPLICATIONS** to the 200 µL of precleared sample. Incubate with gentle agitation for 1 hr. at 4°C.
6) Add 30 µL of 50% protein A agarose beads slurry into the tube. Incubate with gentle agitation for 1 hr. at 4°C.
7) Wash the beads 4 times with 1 mL of IP buffer.
8) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
9) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (10% acrylamide) for electrophoresis.
10) 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.
11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room tempareture.
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
13) Incubate the membrane with 1:500 of Anti-NR1D2 (Rev-erbβ) pAb (PM093) 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.)
14) Wash the membrane with PBS-T (5 min. x 3 times).
15) Incubate the membrane with the 1:20,000 of Rabbit anti-Guinea Pig IgG (H+L) Secondary Antibody, HRP conjugate (Life Technologies; code no. 61-4620) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
16) Wash the membrane with PBS-T (5 min. x 3 times).
17) 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.
18) 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; NIH3T3-3-4)



Immunoprecipitation of mouse Nr1d2 (Rev-erbβ) from NIH3T3-3-4 cells

Lane 1: Input
Lane 2: Normal Guinea Pig IgG (PM067)
Lane 3: Anti-NR1D2 (Rev-erbβ) pAb (PM093)

Immunoblotted with PM093