

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



## Anti-NR1D1 (Rev-erba) pAb

**CODE No.** PM092

**CLONALITY** Polyclonal  
**ISOTYPE** Rabbit Ig, affinity purified  
**QUANTITY** 100  $\mu$ L

**SOURCE** Purified Ig from rabbit 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 1:200-1:1,000 for chemiluminescence detection system  
Immunoprecipitation 2  $\mu$ L/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)

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



## **RELATED PRODUCTS**

### Antibodies

PM092	Anti-NR1D1 (Rev-erb $\alpha$ ) pAb
PM093	Anti- NR1D2 (Rev-erb $\beta$ ) 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
PM035	Normal Rabbit IgG

### Kits

CY-1151	CycLex <sup>®</sup> SIRT1/Sir2 Deacetylase Fluorometric Assay Kit
CY-1152	CycLex <sup>®</sup> SIRT2 Deacetylase Fluorometric Assay Kit
CY-1173	CycLex <sup>®</sup> 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 <sup>+</sup> -Dependent Deacetylase SIRT1
CY-E1152	NAD <sup>+</sup> -Dependent Deacetylase SIRT2
CY-E1173	CaM-kinase II Positive Control

### **SDS-PAGE & Western blotting**

1) Prepare the samples described as below:

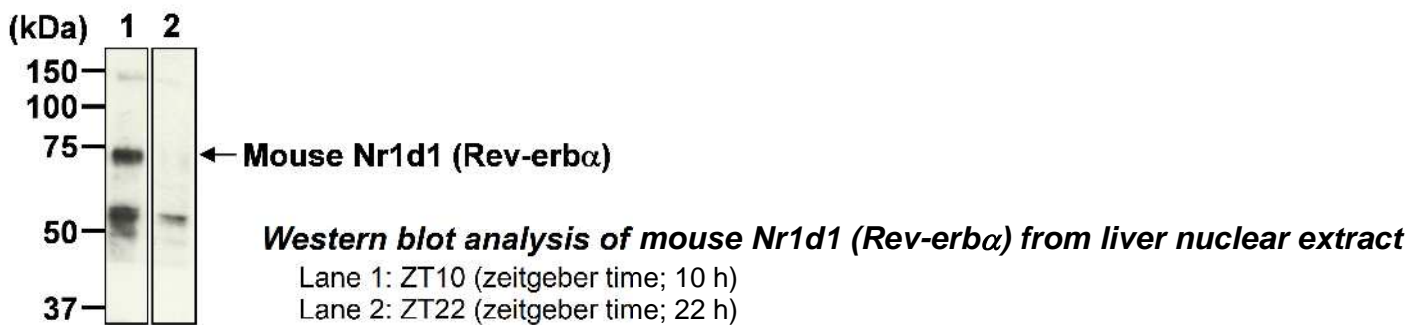
[Tissue] Mix 5  $\mu$ L of mouse liver nuclear extract with 5  $\mu$ L of Laemmli's sample buffer.

[Cell line] Wash  $1 \times 10^7$  cells 3 times with PBS and suspends them in 500  $\mu$ L of Laemmli's sample buffer.

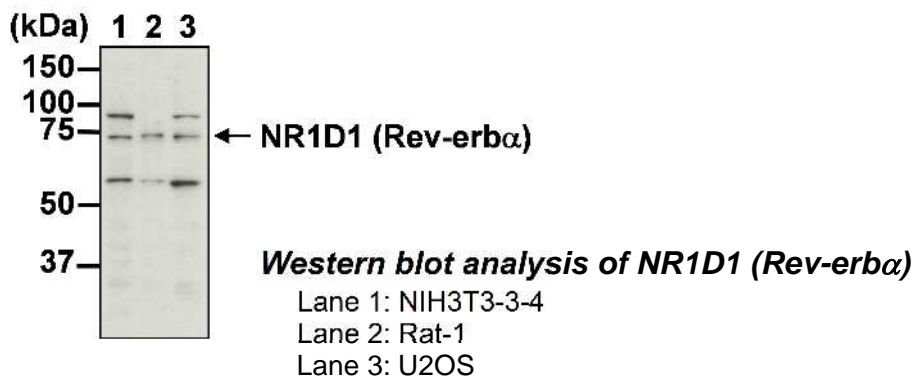
[Transfectant] Wash  $1 \times 10^7$  cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer.

- 2) Boil the samples for 5 min. and centrifuge. Load 10  $\mu$ L of the samples from tissue and cell line or 1  $\mu$ L 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<sup>2</sup> 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.

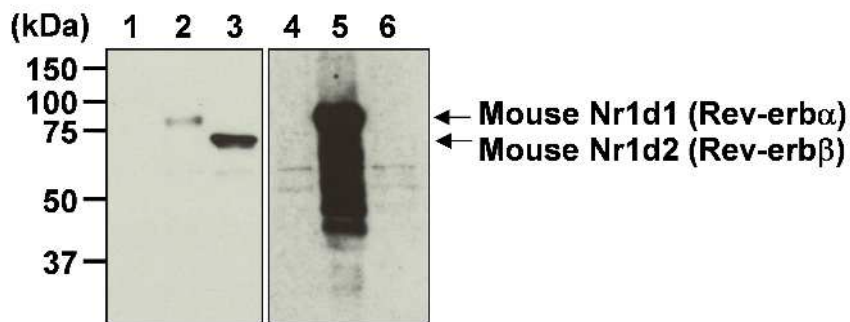
(Positive controls for Western blotting; Mouse liver nuclear extract, NIH3T3-3-4, Rat-1, U2OS and transfectant)



Immunoblotted with Anti-NR1D1 (Rev-erb $\alpha$ ) pAb (PM092), 1:1,000



Immunoblotted with Anti-NR1D1 (Rev-erb $\alpha$ ) pAb (PM092), 1:200



**Western blot analysis of mouse Nr1d1 (Rev-erb $\alpha$ ) 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: 1<sup>st</sup> antibody; Anti-Myc-tag mAb (M047-3), 1  $\mu$ g/mL

2<sup>nd</sup> antibody; Anti-IgG (Mouse) pAb-HRP (330)

Lane 4-6: 1<sup>st</sup> antibody: Anti-NR1D1 (Rev-erb $\alpha$ ) pAb (PM092), 1:1,000

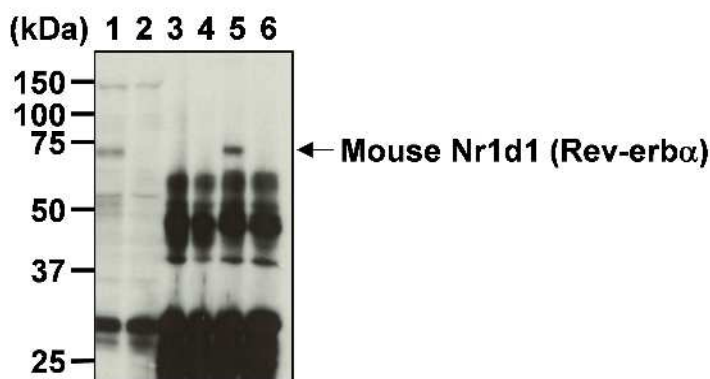
2<sup>nd</sup> antibody: Anti-IgG (Rabbit) pAb-HRP (458)

Exposure time: 15 min.

## **Immunoprecipitation**

- 1) Mix 50  $\mu$ L of mouse liver nuclear extract to 100  $\mu$ L 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  $\text{Na}_3\text{VO}_4$ ] containing appropriate protease inhibitors.
- 2) Add 40  $\mu$ L 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  $\mu$ L of precleared sample (prepared sample from step 3)). Incubate with gentle agitation for 1 hr. at 4°C.
- 5) Mix 30  $\mu$ L 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  $\mu$ L of Laemmli's sample buffer, boil for 5 min. and centrifuge.
- 8) Load 10  $\mu$ L 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<sup>2</sup> 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-erb $\alpha$ ) 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-erb $\alpha$ ) 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-erb $\alpha$ ) pAb (PM092)

Immunoblotted with PM092