

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



Anti-PER2 (Human) pAb

CODE No. PM096

CLONALITY Polyclonal
ISOTYPE Guinea pig Ig, affinity purified
QUANTITY 50 µL

SOURCE Purified Ig from guinea pig serum
FORMURATION TBS containing 50% Glycerol (pH 7.5). 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 1:500 for chemiluminescence detection system
Immunoprecipitation 2 µL/sample

SPECIES CROSS REACTIVITY on WB

| Species | Human | Mouse | Rat | Hamster |
|------------|---|--------------------------------|------------|------------|
| Samples | U2OS cells treated with dexamethasone for 30 hr, transfectant | Liver nuclear extract, NIH/3T3 | Not tested | Not tested |
| Reactivity | + | - | | |

Entrez Gene ID 8864 (Human)

REFERENCES

- 1) Lee, Y., *et al.*, *J. Biol. Chem.* **286**, 7033-7042 (2011)
- 2) Siepka, S. M., *et al.*, *Cold Spring Harb. Symp. Quant. Biol.* **72**, 251-259 (2007)
- 3) Ko, C. H. and Takahashi, J. S., *Hum. Mol. Genet.* **15**, R271-R277 (2006)
- 4) Zheng, B., *et al.*, *Cell* **105**, 683-94 (2001)
- 5) Bae, K., *et al.*, *Neuron* **30**, 525-536 (2001)
- 6) Camacho, F., *et al.*, *FEBS Lett.* **489**, 159-165 (2001)

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



RELATED PRODUCTS

Antibodies

| | |
|----------|--|
| PM096 | Anti-PER2 (Human) pAb |
| PM091 | Anti-Per1 (Mouse) pAb |
| PM083 | Anti-Per2 (Mouse) pAb |
| PM082 | Anti-Cry2 (Mouse) pAb |
| PM081 | Anti-Cry1 (Mouse) pAb |
| PM093 | Anti-NR1D2 (Rev-erb ²) pAb |
| PM092 | Anti-NR1D1 (Rev-erb [±]) pAb |
| D333-3 | Anti-CLOCK (Mouse) mAb (CLSP3) |
| D334-3 | Anti-CLOCK (Mouse) mAb (CLNT1) |
| D349-3 | Anti-CLOCK (Mouse) mAb (CLSP4) |
| D335-3 | Anti-BMAL1 (Mouse) mAb (B1BH2) |
| M225-3 | Anti-NFIL3 (E4BP4) chimeric mAb (42) |
| PM097 | Anti-NFIL3 (E4BP4) pAb |
| 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 |

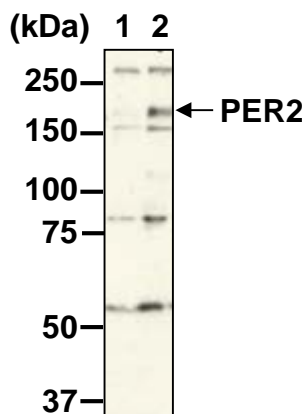
Recombinant proteins (Human, Active)

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

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend them in 500 μ L of Laemmli's sample buffer. Sonicate briefly (up to 30 sec.).
- 2) Boil the sample for 5 min. and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 190 mA for 90 min. 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 control for Western blotting; U2OS cells treated with dexamethasone for 30 hr.)



Western blot analysis of human PER2 from U2OS cells treated with dexamethasone (Dex)

Lane 1: Dex-treated for 18 h

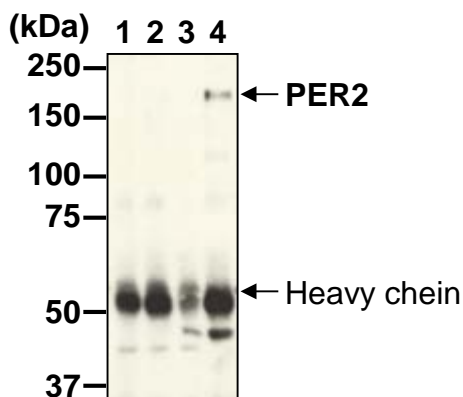
Lane 2: Dex-treated for 30 h

Immunoblotted with Anti-PER2 (Human) pAb (PM096)

Immunoprecipitation

- 1) Wash 1×10^7 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, 1 mM Na_3VO_4] containing appropriate protease inhibitors. Sonicate briefly (up to 10 sec.), then incubate it on ice 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 200 μL of 50% protein G 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 330 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 (7.5% acrylamide) for electrophoresis.
- 10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 190 mA for 90 min. 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) overnight at 4°C.
- 12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 13) 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.)
- 14) Wash the membrane with PBS-T (5 min. x 3 times).
- 15) 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.
- 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; U2OS cells treated with dexamethasone for 30 hr.)



Immunoprecipitation of human PER2 from U2OS cells treated with dexamethasone (Dex)

<Sample>

Lane 1 and 3: Dex-treated for 18 h

Lane 2 and 4: Dex-treated for 30 h

<Antibody>

Lane 1 and 2: Normal Guinea Pig IgG (PM067)

Lane 3 and 4: Anti-PER2 (Human) pAb (PM096)

Immunoblotted with PM096