

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



Anti-Cry2 (Mouse) pAb

CODE No. PM082

CLONALITY Polyclonal
ISOTYPE Guinea pig IgG
QUANTITY 100 µL

SOURCE Purified IgG 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 for chemiluminescence detection system
Immunoprecipitation 2 µL/sample

SPECIES CROSS REACTIVITY on WB

| Species | Human | Mouse | Rat | Hamster |
|------------|------------|--|------------|------------|
| Sample | Not tested | Liver nuclear extract, transfectant | Not tested | Not tested |
| Reactivity | | + | | |

Entrez Gene ID 12953 (Mouse)

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



RELATED PRODUCTS

Antibodies

PM082 Anti-Cry2 (Mouse) pAb
PM081 Anti-Cry1 (Mouse) pAb
PM083 Anti-Per2 (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
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

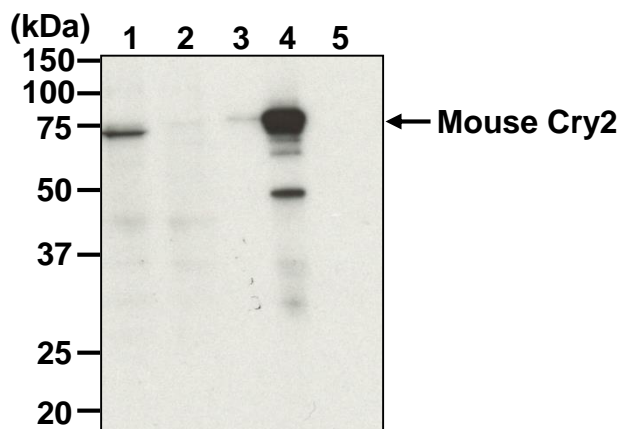
Recombinant proteins (Human, Active)

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 tissue or cell sample described as below:
[Tissue] Mix 10 μ L of mouse liver nuclear extract with 10 μ L of Laemmli's sample buffer.
[Cell] Wash 1×10^7 cells 3 times with PBS and suspends them in 1 mL Laemmli's sample buffer.
- 2) Boil the samples for 5 min. and centrifuge. Load 20 μ L of the tissue sample or 5 μ L of cell sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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 the 1:20,000 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, then incubate it with Immobilon Western Chemiluminescent HRP Substrate (Merck Millipore; code no. WBKLS0100) 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 15 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Mouse liver nuclear extract and transfectant)



Western blot analysis of mouse Cry2

- Lane 1: Mouse liver nuclear extract (ZT24)
- Lane 2: Cry1/Cry2 double knockout mouse liver nuclear extract (ZT24)
- Lane 3: Mouse Cry1/HEK293T
- Lane 4: Mouse Cry2/HEK293T
- Lane 5: HEK293T

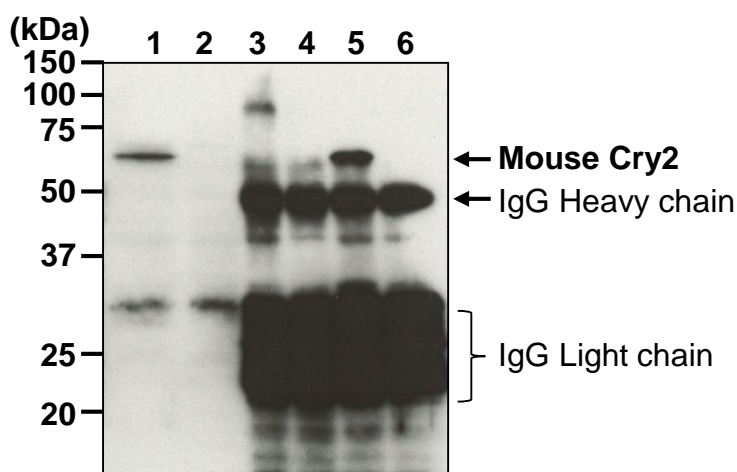
Immunoblotted with Anti-Cry2 (Mouse) pAb (PM082)

Cry1/Cry2 double knockout mouse was kindly provided by Dr. Toshiyuki Hamada.
(Hokkaido University Graduate School of Medicine)

Immunoprecipitation

- 1) Add 30 μL of 50% protein A agarose beads slurry resuspended in 100 μL of ice-cold 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 into the 50 μL of mouse liver nuclear extract. Incubate it at 4°C with rotating 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 (precleared sample).
- 3) Add primary antibody as suggested in the **APPLICATIONS** to the 150 μL of precleared sample (prepared sample from step 2)). Incubate with gentle agitation for 1 hr. at 4°C.
- 4) Mix 30 μL of 50% protein A agarose beads slurry into the tube. Incubate with gentle agitation for 1 hr. at 4°C.
- 5) Wash the beads 4 times with 1 mL of IP buffer.
- 6) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
- 7) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 8) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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.
- 9) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 11) Incubate the membrane with 1:200 of Anti-Cry2 (Mouse) pAb (MBL; code no. PM082) 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.)
- 12) Wash the membrane with PBS-T (5 min. x 3 times).
- 13) Incubate the membrane with the 1:20,000 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.
- 14) Wash the membrane with PBS-T (5 min. x 3 times).
- 15) Wipe excess buffer on the membrane, then incubate it with Immobilon Western Chemiluminescent HRP Substrate (Merck Millipore; code no. WBKLS0100) for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 16) Expose to an X-ray film in a dark room for 15 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Mouse liver nuclear extract)



Immunoprecipitation of mouse Cry2 from liver nuclear extracts

<Sample>

Lane 1, 3, 5: Mouse liver nuclear extract (ZT24)

Lane 2, 4, 6: Cry1/Cry2 double knockout mouse
liver nuclear extract (ZT24)

Lane 1, 2: Input (tissue lysate)

Lane 3, 4: Normal Guinea Pig IgG (PM067)

Lane 5, 6: Anti-Cry2 (Mouse) pAb (PM082)

Immunoblotted with PM082

Cry1/Cry2 double knockout mouse was kindly provided by Dr. Toshiyuki Hamada. (Hokkaido University Graduate School of Medicine)