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

MBL
A JSR Life Sciences Company

Anti-Cry1 (Mouse) pAb

CODE No. PM081

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

Western blotting	1:1,000
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 12952 (Mouse)

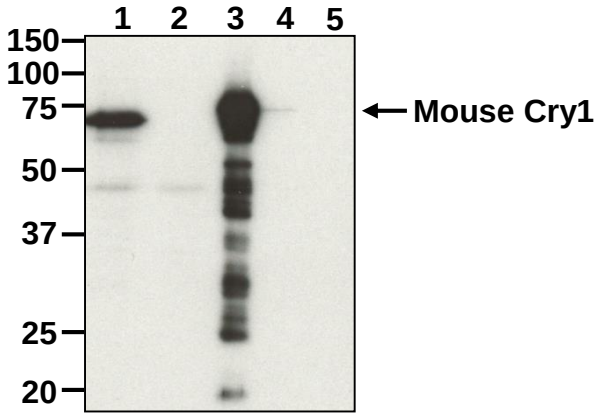
For more information, please visit our web site **https://ruo.mbl.co.jp/**.

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

SDS-PAGE & Western blotting

1) Prepare the tissue or cell sample described as below:
   [Tissue] Mix 10 μL of mouse liver nuclear extract with 10 μL of Laemmli's sample buffer.
   [Cell] Wash 1 x 10^7 cells 3 with PBS and suspends them in 1 mL Laemmli's sample buffer.
2) Boil the samples for 5 min. and centrifuge. Load 20 μL of the tissue sample or 5 μL 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/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
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).
8) Incubate the membrane with the 1:20,000 Rabbit anti-Guinea Pig IgG (H+L) Secondary Antibody, HRP conjugate (Thermo Fisher Scientific; 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).
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 blotting analysis of mouse Cry1

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

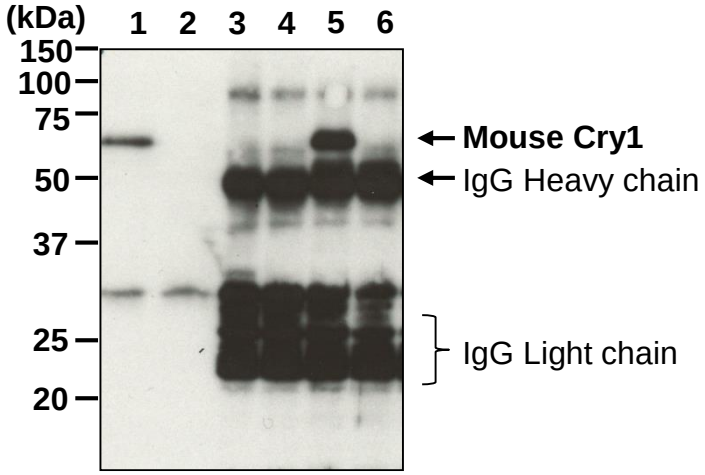
Immunoblotted with Anti-Cry1 (Mouse) pAb (MBL, code no. PM081)

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 (pH 7.8), 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/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
11) Incubate the membrane with 1:1,000 of Anti-Cry1 (Mouse) pAb (MBL; code no. PM081) 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).
13) Incubate the membrane with the HRP conjugate Rabbit anti-Guinea Pig IgG (H+L) Secondary Antibody (Thermo Fisher Scientific; code no. 61-4620) , diluted 1:20,000 in 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).
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 Cry1 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 (MBL, code no. PM067)
Lane 5, 6: Anti-Cry1 (Mouse) pAb (MBL, code no. PM081)
Immunoblotted with PM081

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