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

Anti-Per2 (Mouse) pAb

CODE No. PM083

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified Ig from rabbit serum
FORMULATION 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
Immunoprecipitation	2 µL/sample
Immunohistochemistry	Can be used.

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Samples	Transfectant	Liver nuclear extracts, transfectant	Not tested	Not tested
Reactivity	-	+		

Entrez Gene ID 18627 (Mouse)

REFERENCE

1) Wang, Q., *et al., Int. J. Mol. Sci.* **19**, E3134 (2018) [WB]
2) Wu, Y., *et al*., *Cell Metab*. **25**, 73-85 (2017) [WB]

RELATED PRODUCTS

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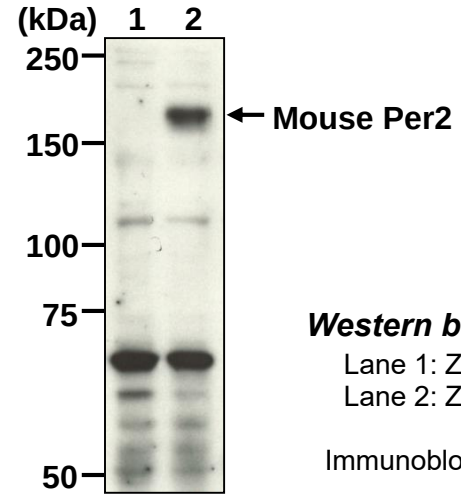
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

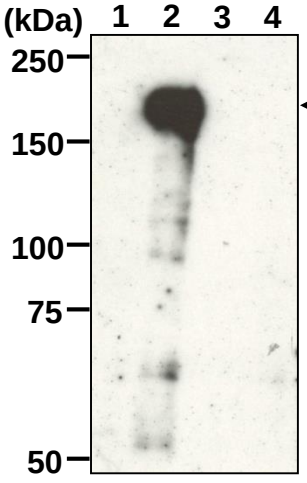
1) Mix 5 μL of mouse liver nuclear extract with 5 μL of Laemmli's sample buffer.
2) Boil the samples for 5 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 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 1:10,000 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.
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 30 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Mouse liver nuclear extract (ZT18))



Western blot analysis of mouse Per2 in liver nuclear extracts
Lane 1: ZT6 (zeitgeber time; 6 h)
Lane 2: ZT18 (zeitgeber time; 18 h)

Immunoblotted with Anti-Per2 (Mouse) pAb (PM083)



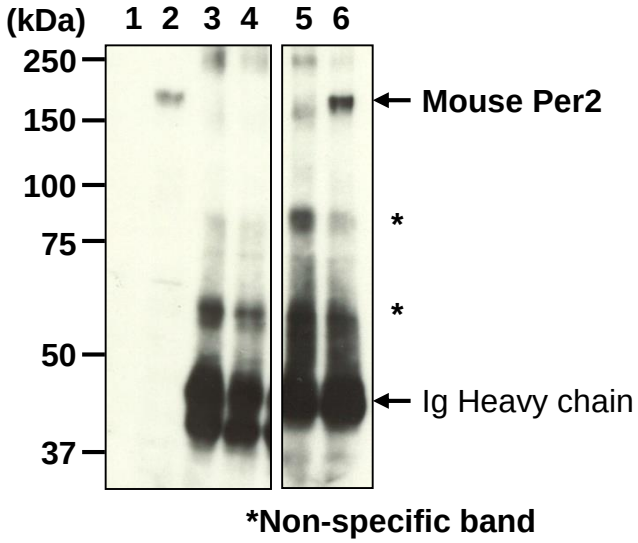
Western blot analysis of mouse Per2 transfectant
Lane 1: HEK293T
Lane 2: Mouse Per2/HEK293T
Lane 3: Mouse Per1/HEK293T
Lane 4: Human Per2/HEK293T

Immunoblotted with Anti-Per2 (Mouse) pAb (PM083)

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 extracts. 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 (7.5% acrylamide) for electrophoresis.
8) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1.5 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-Per2 (Mouse) pAb (MBL; code no. PM083) 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 1:10,000 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.
14) Wash the membrane with PBS-T (5 min. x 3 times).
15) Wipe excess buffer on the membrane, 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 a plastic wrap.
16) Expose to an X-ray film in a dark room for 5 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Mouse liver nuclear extracts (ZT16))



Immunoprecipitation of mouse Per2 in liver nuclear extracts

<Sample>
Lane 1, 3, 5: ZT4 (zeitgeber time; 4 h)
Lane 2, 4, 6: ZT16 (zeitgeber time; 16 h)

Lane 1, 2: Input (tissue lysate)
Lane 3, 4: Normal Rabbit IgG (PM035)
Lane 5, 6: Anti-Per2 (Mouse) pAb (PM083)

Immunoblotted with PM083