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

Anti-Per1 (Mouse) pAb

CODE No. PM091

CLONALITY Polyclonal
ISOTYPE Guinea pig IgG
QUANTITY 100 µL

SOURCE Purified IgG from guinea pig 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	1:1,000
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 18626 (Mouse)

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RELATED PRODUCTS

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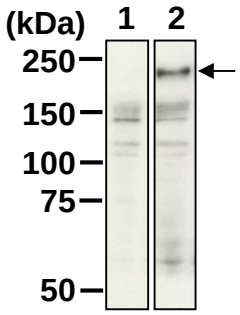
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

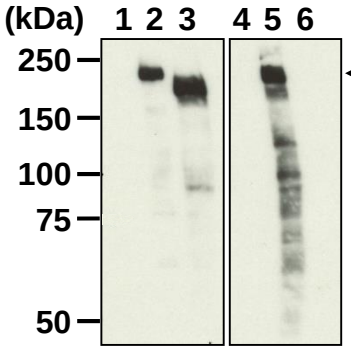
1) Prepare the tissue or cell sample described as below:
   [Tissue] Mix 10 μL of mouse liver nuclear extract with 10 μL of Laemmli's sample buffer.
   [Cell] Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 5 min. and centrifuge. Load 10 μg of the tissue sample or 10 μL of cell 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 1:20,000 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 1 min. 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 Per1 from liver nuclear extract
Lane 1: ZT6 (zeitgeber time; 6 h)
Lane 2: ZT18 (zeitgeber time; 18 h)

Immunoblotted with Anti-Per1 (Mouse) pAb (PM091)



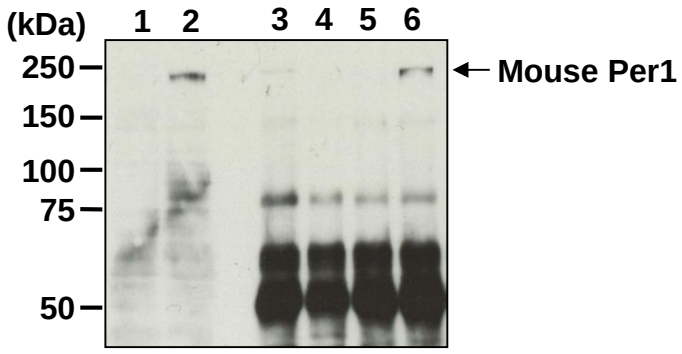
Western blot analysis of mouse Per1 from HEK293T transfectant
Lane 1, 4: HEK293T
Lane 2, 5: Myc-tagged mouse Per1/HEK293T
Lane 3, 6: Myc-tagged mouse Per2/HEK293T

<Immunoblot>
Lane 1-3: 1st antibody; Anti-Myc-tag mAb (M047-3)
2nd antibody; Anti-IgG (Mouse) pAb-HRP (330)
Lane 4-6: 1st antibody: Anti-Per1 pAb (PM091)
2nd antibody: Rabbit anti-Guinea Pig IgG (H+L) Secondary Antibody, HRP conjugate (Life Technologies; code no. 61-4620)

Immunoprecipitation

1) Mix 650 μg of mouse liver nuclear extract to 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 (final volume: 487.5 μL).
2) Add 200 μL 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 150 μL of the precleared sample (prepared sample from step 3)). Incubate with gentle agitation for 1 hr. at 4°C.
5) Mix 30 μL 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 20 μL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
8) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
9) 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.
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-Per1 (Mouse) pAb (MBL; code no. PM091) 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 1:20,000 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.
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 Per1 from liver nuclear extract

<Sample>
Lane 1, 3, 5: ZT6 (zeitgeber time; 6 h)
Lane 2, 4, 6: ZT18 (zeitgeber time; 18 h)

Lane 1, 2: Input
Lane 3, 4: Normal Guinea Pig IgG (PM067)
Lane 5, 6: Anti-Per1 (Mouse) pAb (PM091)

Immunoblotted with PM091