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Anti-CLOCK (Mouse) mAb

CODE No. D333-3

CLONALITY Monoclonal
CLONE CLSP3
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Ser/Pro-rich region (Ser^{377}-Glu^{556}) of mouse CLOCK
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.

APPLICATION-CONFIRMED
Western blotting 1 µg/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	Not tested	Liver nuclear extract	Not tested	Not tested
Reactivity		+		

Entrez Gene ID 12753 (Mouse)

REFERENCES
1) Yoshitane, H., *et al.*, *EMBO Rep.* **13**, 455-461 (2012) [WB]
2) Yoshitane, H., *et al.*, *Mol Cell Biol.* **29**, 3675-3686 (2009) [WB]

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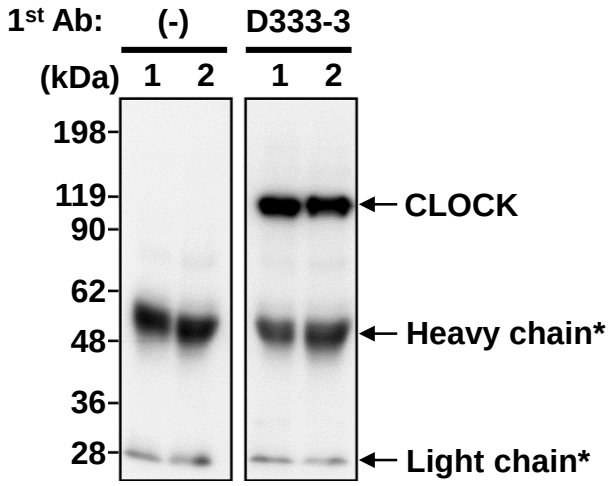
PROTOCOL

SDS-PAGE & Western blotting

1) Mix 10 μL of Mouse liver nuclear extract with 10 μL of Laemmli's sample buffer.
2) Boil the sample for 3 min. and centrifuge. Load 20 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 300 mA for 1 hr. in a wet 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 1% skimmed milk (in TBS, pH 7.2) for 1 hr. at room temperature.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in TBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at 37°C, 2 hr. at room temperature or overnight at 4°C. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane 3 times for 2 min., 5 min. and 10 min. each with 1% skimmed milk (in TBS, pH 7.2).
7) Incubate the membrane with the 1:5,000 anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in TBS, pH 7.2) for 2 hr. at room temperature or overnight at 4°C.
8) Wash the membrane 3 times for 2 min., 5 min. and 10 min. each with TBS-T [0.05% Tween-20 in TBS].
9) Wash the membrane 1 time for 2 min. with TBS.
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 controls for Western blotting; Mouse liver nuclear extracts)



*The heavy/light chains derived from IgG in the samples.
(These bands are detected depending on a sample.)

Western blotting analysis of mouse CLOCK from liver nuclear extracts

1: ZT6 (zeitgeber time; 6 h)
2: ZT18 (zeitgeber time; 18 h)

Immunoblotted with Anti-CLOCK (Mouse) mAb (D333-3)

Data were provided by Mr. Kentaro Hirose, Dr. Hikari Yoshitane, Ph.D. and Dr. Yoshitaka Fukada, Ph.D. (Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo)