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Not for use in diagnostic procedures.

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Anti-BMAL1 mAb

CODE No. D335-3

CLONALITY Monoclonal
CLONE B1BH2
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN bHLH domain (Ala^{73}-Ala^{128}) of mouse BMAL1
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.

APPLICATION-CONFIRMED
Western blotting 1-10 µg/mL

APPLICATION-REPORTED
Chromatin Immunoprecipitation Reference 2)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	U2OS	Liver nuclear extract, NIH/3T3	Not tested	Not tested
Reactivity	+	+		

Entrez Gene ID 406 (Human), 11865 (Mouse)

REFERENCES

1) Kon, N., *et al.*, *Genes Dev.* **28**, 1101-1110 (2014) [WB]
2) Yoshitane, H., *et al.*, *Mol Cell Biol.* **34**, 1776-1787 (2014) [ChIP]
3) Yoshitane, H., *et al.*, *EMBO Rep.* **13**, 455-461 (2012) [WB]
4) Yoshitane, H., *et al.*, *Mol Cell Biol.* **29**, 3675-3686 (2009) [WB]

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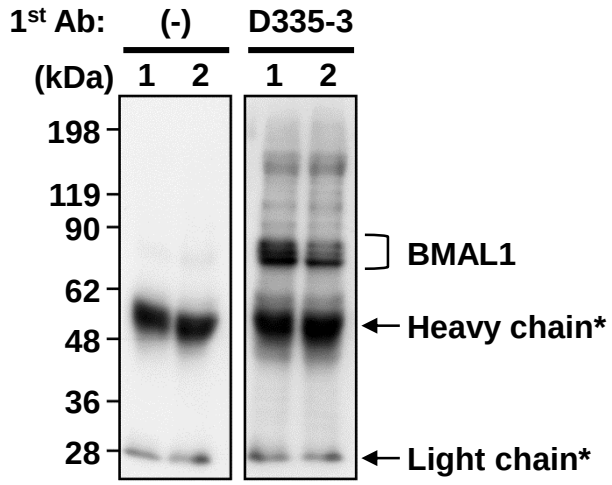
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 1:5,000 of 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, U2OS and NIH/3T3)



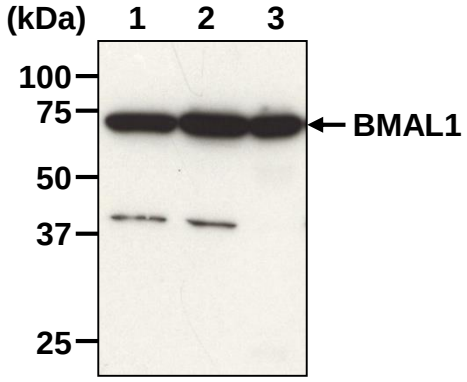
*The heavy/light chains derived from IgG in the samples.
(These bands are detected depending on a sample.)

Western blotting analysis of mouse BMAL1 from liver nuclear extracts

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

Immunoblotted with Anti-BMAL1 mAb (D335-3)

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)



Western blotting analysis of BMAL1

Lane 1: NIH/3T3
Lane 2: U2OS
Lane 3: Mouse liver nuclear extract, ZT12 (zeitgeber time; 12 h)

Immunoblotted with Anti-BMAL1 mAb (D335-3)