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Not for use in diagnostic procedures.

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Anti-CLOCK mAb

CODE No.	D349-3
CLONALITY	Monoclonal
CLONE	CLSP4
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	MBP-Ser/Pro-rich region (Ser^{377}-Glu^{556}) of mouse Clock
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 µg/mL for chemiluminescence detection system
Immunoprecipitation	2 µg/sample
Immunohistochemistry	Can be used.

APPLICATION-REPORTED

Chromatin Immunoprecipitation (ChIP) Reference 2)

SPECIES CROSS REACTIVITY on WB

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

Entrez Gene ID 9570 (Human), 12753 (Mouse)

REFERENCES

1) Tsurudome, Y., *et al., Sci. Rep.* **8**, 9072 (2018) [WB]
2) Yoshitane, H., *et al.*, *Mol. Cell Biol.* **34**, 1776-1787 (2014) [ChIP]
3) Yoshitane, H., *et al.*, *Mol Cell Biol.* **29**, 3675-3686 (2009) [IP]

RELATED PRODUCTS

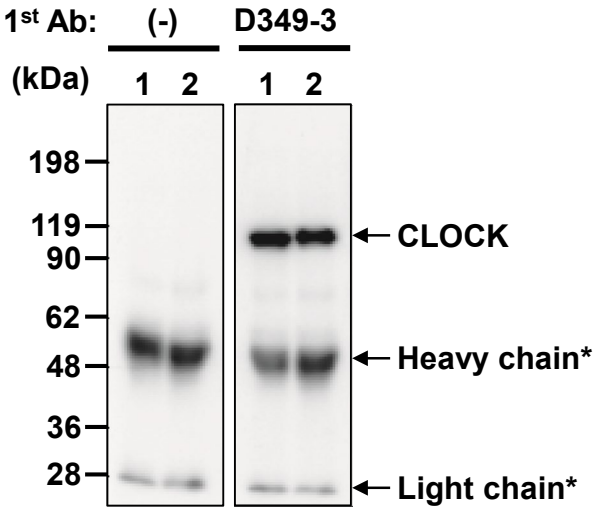
For more information, please visit our web site https://ruo.mbl.co.jp/.

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

SDS-PAGE & Western blotting

1) 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 (8% 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% MeOH). 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 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 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)



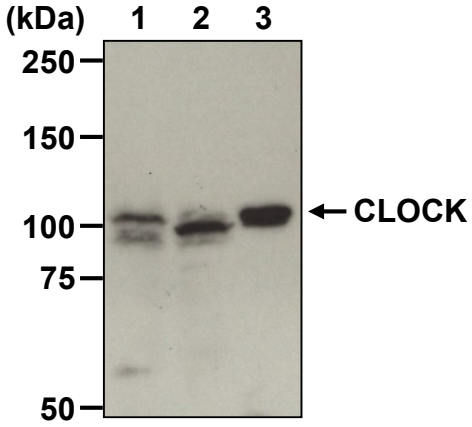
*The heavy/light chains derived from IgG in the samples.
(These bands are detected depending on a sample.)

Western blot analysis of mouse CLOCK from liver nuclear extracts

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

Immunoblotted with Anti-CLOCK (Mouse) mAb (D349-3)

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

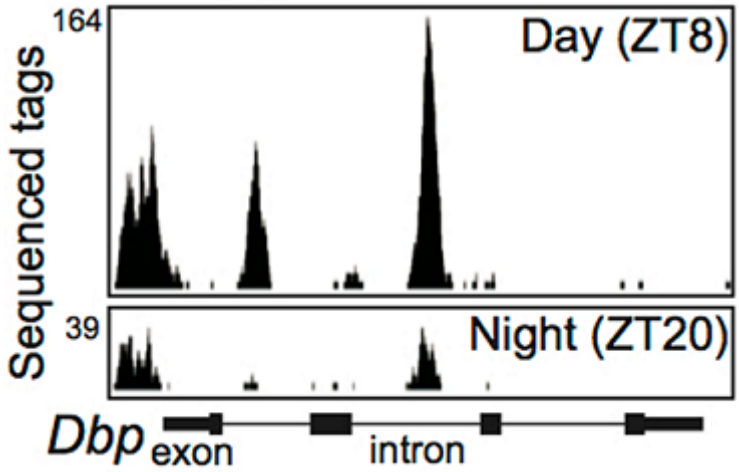


Western blot analysis of CLOCK

Lane 1: NIH/3T3
Lane 2: U2OS
Lane 3: Mouse liver nuclear extract, ZT6 (zeitgeber time; 6 h)

Immunoblotted with Anti-CLOCK (Mouse) mAb (D349-3)

Chromatin Immunoprecipitation (under evaluation)



Sequencing analysis of immunoprecipitated DNA using Dbp locus

Sample: Mouse liver nuclear fraction
Antibody: Anti-CLOCK (Mouse) mAb (D349-3; clone CLSP4), 1 μg/20 μL protein G agarose
Sequencing: Genome Analyzer IIx (Illumina)

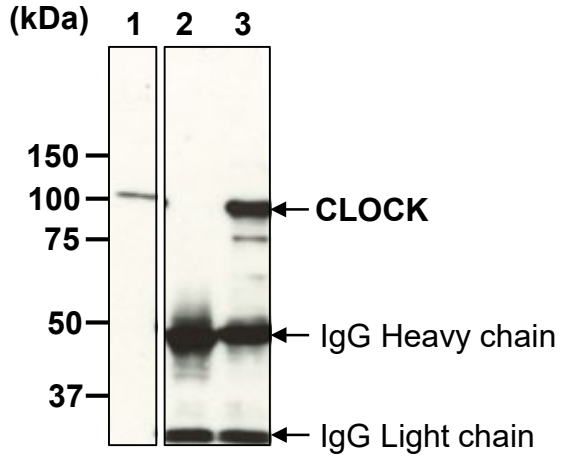
Data were kindly provided by Dr. Hikari Yoshitane, and Dr. Yoshitaka Fukada. (Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo)

Reference: Yoshitane, H., *et al., Mol. Cell Biol*. **34**, 1776-1787 (2014) [PMID: 24591654]

Immunoprecipitation

1) Add 30 µL of 50% protein G 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 G agarose beads slurry into the tube. Incubate with gentle agitation for 1 hr. at 4°C.
5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
6) Resuspend the agarose with 1 mL of IP buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
8) Repeat steps 5)-7) 4 times. Wash the beads 4 times with 1 mL of IP buffer.
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
10) Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
11) 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.
12) To reduce nonspecific binding, soak the membrane in 1% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
13) Incubate the membrane with 1 µg/mL of Anti-CLOCK (Mouse) mAb (MBL; code no. D333-3) 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.)
14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
15) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
16) Wash the membrane with PBS-T (5 min. x 3 times)
17) 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 plastic wrap.
18) Expose to an X-ray film in a dark room for 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Immunoprecipitation; Mouse liver nuclear extracts)



Immunoprecipitation of mouse CLOCK from liver nuclear extracts

Sample: Mouse liver nuclear extract (ZT6)
Lane 1: Input (precleared with protein G)
Lane 2: IP with Mouse IgG1 (M075-3)
Lane 3: IP with Anti-CLOCK (Mouse) mAb (D349-3; clone CLSP4)

Immunoblotted with Anti-CLOCK (Mouse) mAb (D333-3; clone CLSP3)