

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



Anti-CLOCK mAb

CODE No.	D349-3
CLONALITY	Monoclonal
CLONE	CLSP4
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 μ L, 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

<u>Western blotting</u>	1 μ g/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	2 μ g/sample
<u>Immunohistochemistry</u>	Can be used.

APPLICATION-REPORTED

<u>Chromatin Immunoprecipitation (ChIP)</u>	Reference 2)
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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]

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

RELATED PRODUCTS

Antibodies

D333-3	Anti-CLOCK (Mouse) mAb (CLSP3)
D349-3	Anti-CLOCK mAb (CLSP4)
D334-3	Anti-CLOCK (Mouse) mAb (CLNT1)
D335-3	Anti-BMAL1 (Mouse) mAb (B1BH2)
D361-3	Anti-BMAL1 mAb (2F11)
PM087	Anti-Chrono (Mouse) pAb
PM092	Anti-NR1D1 (Rev-erb α) pAb
PM093	Anti-NR1D2 (Rev-erb β) pAb
PM091	Anti-Per1 (Mouse) pAb
PM083	Anti-Per2 (Mouse) pAb
PM096	Anti-PER2 (Human) pAb
PM081	Anti-Cry1 (Mouse) pAb
PM082	Anti-Cry2 (Mouse) pAb
PM079	Anti-DBP (Mouse) pAb
M225-3	Anti-NFIL3 (E4BP4) chimeric mAb (42)
PM097	Anti-NFIL3 (E4BP4) pAb
PM075	Anti-GNAT2 (Zebrafish) pAb
CY-P1016	Anti-SIRT1 pAb

Kits

CY-1151V2	CycLex [®] SIRT1/Sir2 Deacetylase Fluorometric Assay Kit Ver.2
CY-1152V2	CycLex [®] SIRT2 Deacetylase Fluorometric Assay Kit Ver.2
CY-1173	CycLex [®] CaM-kinase II Assay Kit

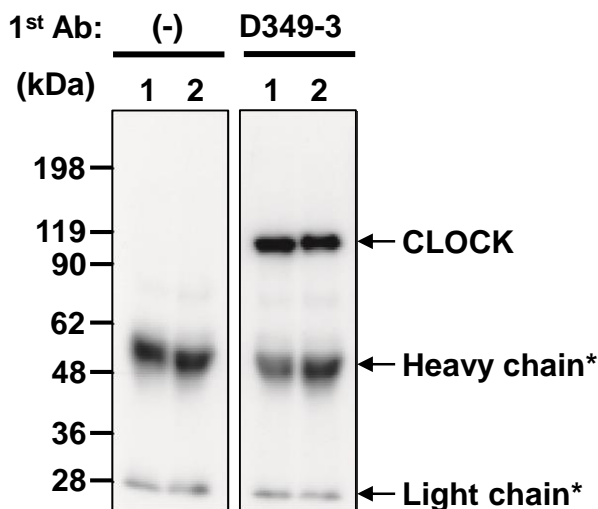
Recombinant proteins (Human, Active)

CY-E1151	NAD ⁺ -Dependent Deacetylase SIRT1
CY-E1152	NAD ⁺ -Dependent Deacetylase SIRT2
CY-E1173	CaM-kinase II Positive Control

SDS-PAGE & Western blotting

- 1) Mix 10 μ L of Mouse liver nuclear extract with 10 μ L of Laemmli's sample buffer.
- 2) Boil the sample for 3 min. and centrifuge. Load 20 μ L 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.)

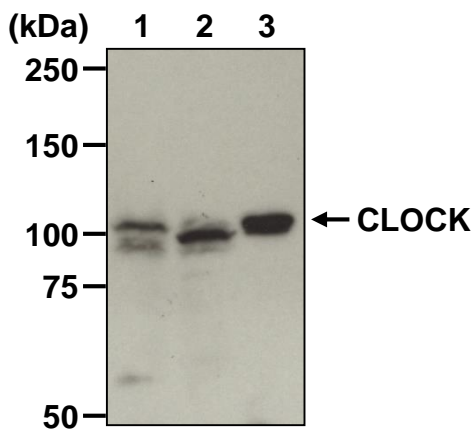
Western blot analysis of mouse CLOCK from liver nuclear extracts

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

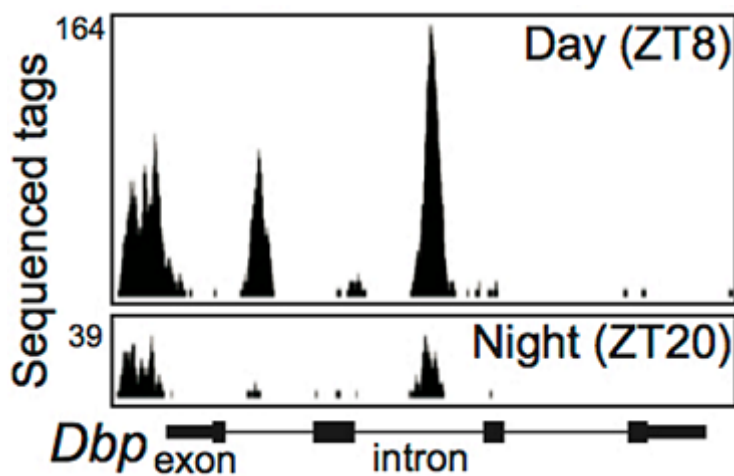
Lane 1: NIH/3T3

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 μ g/20 μ L protein G agarose

Sequencing: Genome Analyzer IIx (Illumina)

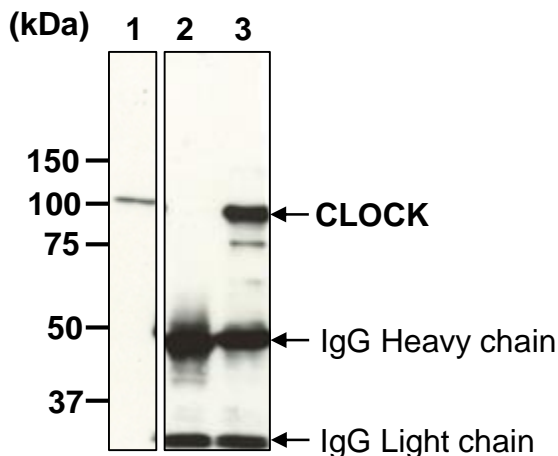
Data were kindly provided by Dr. Hikari Yoshitane, and Dr. Yoshitaka Fukada. (Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo)

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

Immunoprecipitation

- 1) Add 30 μ L of 50% protein G agarose beads slurry resuspended in 100 μ L 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 μ L 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 μ L of precleared sample (prepared sample from step 2). Incubate with gentle agitation for 1 hr. at 4°C.
- 4) Mix 30 μ L 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 μ L of Laemmli's sample buffer, boil for 5 min. and centrifuge.
- 10) Load 20 μ L 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/cm² 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 μ g/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)