Anti-CLOCK Antibody [JA12-33]

ET1704-82

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 95 kDa
Clone number:	JA12-33

Description: Biological timepieces called circadian clocks are responsible for the regulation of hormonal rhythms, sleep cycles and other behaviors. The superchiasmatic nucleus (SCN), which is located in the brain, was the first mammalian circadian clock to be discovered. Clock, a member of the Basic-helix-loop-helix-psp (bHLH-PAS) family of transcription factors, has also been identified as having circadian function. Mutations within the clock gene have been shown to increase the length of the endogenous period and To contain a loss of rhythmicity of circadian oscillations. Clock contains a DNA-binding domain, a protein dimerization domain and a glutamine-rich C-terminal region, which indicates transactivation ability. It has been speculated that Clock may regulation circadian rhythmicity in combination with Other proteins such as Per. Per is also a PAS-domain containing protein that exhibits circadian function. Highest expression of Clock is seen in the hypothalamus and the eye.

Immunogen: Synthetic peptide within Human CLOCK aa 140-189 / 846.

Positive control: HeLa cell lysate, HEK-293 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, Human brain tissue lysate, human colon tissue, mouse colon tissue, rat colon tissue, PC-12.

Subcellular location: Nucleus, Cytosol, Cytoplasm.

Database links: SwissProt: O15516 Human | O08785 Mouse | Q9WVS9 Rat

Recommended Dilutions:

WB	1:500-1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:1,000
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

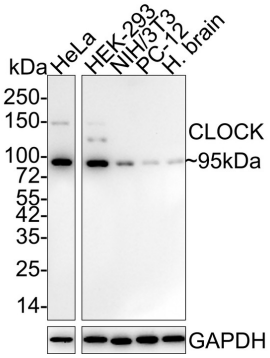

Fig1: Western blot analysis of CLOCK on different lysates with Rabbit anti-CLOCK antibody (ET1704-82) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: HEK-293 cell lysate (10 µg/Lane)
Lane 3: NIH/3T3 cell lysate (10 µg/Lane)
Lane 4: PC-12 cell lysate (10 µg/Lane)
Lane 5: Human brain tissue lysate (20 µg/Lane)

Predicted band size: 95 kDa
Observed band size: 95 kDa

Exposure time: 1 minute;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1704-82) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

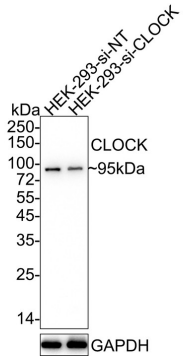

Fig2: Western blot analysis of CLOCK on different lysates with Rabbit anti-CLOCK antibody (ET1704-82) at 1/5,000 dilution.

Lane 1: HEK-293-si NT cell lysate
Lane 2: HEK-293-si CLOCK cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 95 kDa
Observed band size: 95 kDa

Exposure time: 50 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1704-82) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

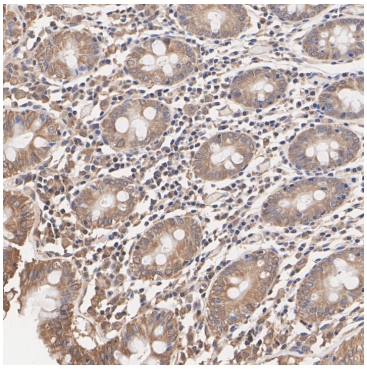

Fig3: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-CLOCK antibody (ET1704-82) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-82) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

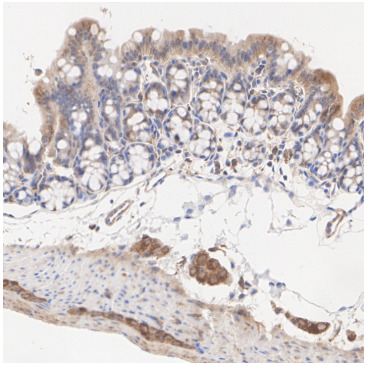

Fig4: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-CLOCK antibody (ET1704-82) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-82) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

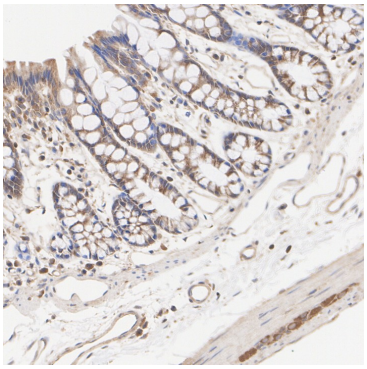

Fig5: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-CLOCK antibody (ET1704-82) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-82) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

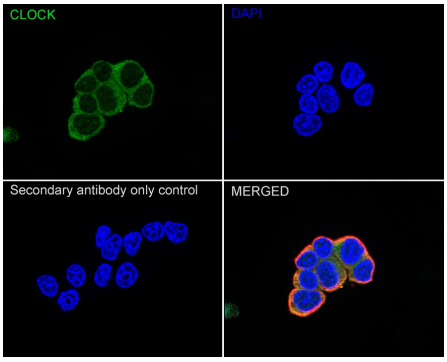

Fig6: Immunocytochemistry analysis of PC-12 cells labeling CLOCK with Rabbit anti-CLOCK antibody (ET1704-82) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CLOCK antibody (ET1704-82) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

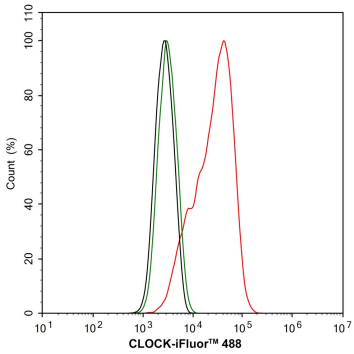

Fig7: Flow cytometric analysis of PC-12 cells labeling CLOCK.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1704-82, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Matsumura R, et al. The mammalian circadian clock protein period counteracts cryptochrome in phosphorylation dynamics of circadian locomotor output cycles kaput (CLOCK). J Biol Chem. 2014
2. Li X, et al. Circadian locomotor output cycles kaput affects the proliferation and migration of breast cancer cells by regulating the expression of E-cadherin via IQ motif containing GTPase activating protein 1. Oncol Lett. 2018