

Product Specification Sheet

Clock Antibodies

Cat. CLO11-S	Rabbit Anti-Mouse Clock antiserum # 1	SIZE: 100 ul
Cat. CLO11-A	Rabbit Anti-Mouse Clock IgG #1 (aff pure)	SIZE: 100 ug
Cat. CLO11-P	Mouse Clock Control/blocking peptide #1	SIZE: 100 ug

Several endogenous factors have been linked to rhythmicity or circadian behavior of living organisms. In *Drosophila*, the genes *period* (**dPer**) and *timeless* (*tim*), and in *Neurospora* *frequency* (*freq*), have been proposed to be responsible for their circadian rhythm. Recently human and mouse genes encoding a basic helix-loop-helix (bHLH) and Per-ARNT-Sim (PAS)-domain with significant similarity to the *Drosophila* Period have been reported. The cDNA sequences of hPER and mPer1 (also named *RIGUI*) are predicted to encode for proteins of length 1290 and 1291 amino acids respectively. Homologues of mPer1 designated **Per 2** (1257 aa) and **Per3** (1113 aa) have also been cloned. Both Per1 and Per2 levels show circadian rhythm in the SCN and eyes. It has been suggested that mPer regulates neuronal activity in the SCN. Using genetic approach, a single mutation (A to T in the **Clock** gene affects circadian rhythmicity in mice. Clock has been mapped to chromosome 5. Mouse *Clock* encodes a transcription factor, a single polypeptide chain of 855 aa (predicted calculated mol wt ~97 kDa; pI 6.52; hClock, 846 aa). Clock is abundantly expressed in brain (SCN, pyriform cortex, hippocampus) as well as in other tissues (eye, total brain, testes, ovaries, liver, heart, lung, and kidney). Although, *Clock* is constitutively expressed (not rhythmic) in the SCN, it may still be an important component of circadian machinery.

Storage

Short-term: unopened, undiluted liquid vials at -200C and powder at 4oC or -20oC..

Long-term: at -20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at -20oC or below.

Shipping: 4oC for solutions and room temp for powder

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure using Chemiluminescence technique).

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence: We recommend the use of affinity purified antibody at 2-20 ug/ml (see refs 2 on this antibody).

Source of Antigen and Antibodies

Antigen	18-aa peptide of mouse Clock (protein accession #, refs 1) ; Designated (CLO11-P or control peptide) conjugated to KLH; epitope location ~ mapping downstream of the serine rich region of mouse Clock
Ab Host/type	Rabbit, Polyclonal antiserum # CLO11-S and IgG, purified over antigen-agarose (Cat # CLO11-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	Cat # 20009-1, Rabbit (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as -ve control

Specificity & Cross-reactivity

The mouse CLO11-P peptide sequence is 94% conserved in rat , human and 81% in chicken Clock. No significant homology is seen with dPER or other known proteins. Antibody cross-reactivity with Per1 from other species is not known. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at the web site).

General References: (1) Antoch MP et al (1997) *Cell* 89, 655-667; King, D. P. et al., (1997) *Cell*, **89**, 641-653; Gekakis N et al (1998) *Science* 280, 1564-1569; Stevens TD et al (1999) *Genomics* 57, 189-200

Citations of ADI's Per1 antibodies

Gustincich S, 2004, *Proc. Natl. Acad. Sci.* 101 , 5069-5074
Curtis AM, 2003, *J. Biol. Chem.* 279, 7091-7097
Allen GC, 2004, *Neuroscience*, 127, 4, , 989-999
Zanello SB., 2000, *J. Invest. Dermatol.* 115: 757-760
Takahashi J S, 1999, *Science* 24; 285: 2076-2077
Allada R, 2003, *EMBO J.*, 22: 3367 - 3375
Maywood ES, 2002, *Eur. J. Neurosci.* 15, 1, 216-220,
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*This product is for *in vitro* research use only.

Related material available from ADI

Anti-Mouse/human Per1-3, Clock, MOP3-4; *Drosophila* Per, dClock, dBMAL, CRY1 and CRY2.

CLO11-S-A-P 71209A

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)

100ul solution lyophilized powder
Supplied 0.05% azide, **Reconstitute** powder in 100 ul PBS

Affinity pure IgG

100 ug/100ul solution lyophilized powder
Supplied in **Buffer:** PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide

100 ug/100 ul solution lyophilized powder
Supplied in **Buffer:** PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

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