abcam

Product datasheet

Anti-BMAL1 antibody ab3350

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Overview

Product name Anti-BMAL1 antibody

Description Rabbit polyclonal to BMAL1

Host species Rabbit

Specificity Detects BMAL 1 / aryl hydrocarbon nuclear translocator 3 (ARNT 3) from hamster and mouse

tissues as well as recombinant human BMAL 1.

Tested applications Suitable for: WB, ICC

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Horse, Chicken, Syrian hamster

Immunogen Synthetic peptide corresponding to Mouse BMAL1 aa 582-594.

Sequence:

DMIDNDQGSSSPS

(Peptide available as ab4959)

Run BLAST with
Run BLAST with

Positive control WB: human U251 and U87-MG, mouse NIH-3T3 cell lines; human SH-SY5Y, U-87 MG, U-2 OS,

HeLa, HEK293, and JURKAT cells, and rat brain tissue; ICC: SH-SY5Y cells

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, PBS

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab3350 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★ ★ ★ ★ (1)	1/200. Predicted molecular weight: 69.4 kDa.Can be blocked with BMAL1 peptide (ab4959). This antibody detects a protein which corresponds to the product of a hamster BMAL 1 fusion construct overexpressed in E. coli (110 kDa band)as well as recombinant human BMAL1.
ICC		Use a concentration of 2 µg/ml.

Target

Function

Transcriptional activator which forms a core component of the circadian clock. The circadian clock, an internal time-keeping system, regulates various physiological processes through the generation of approximately 24 hour circadian rhythms in gene expression, which are translated into rhythms in metabolism and behavior. It is derived from the Latin roots 'circa' (about) and 'diem' (day) and acts as an important regulator of a wide array of physiological functions including metabolism, sleep, body temperature, blood pressure, endocrine, immune, cardiovascular, and renal function. Consists of two major components: the central clock, residing in the suprachiasmatic nucleus (SCN) of the brain, and the peripheral clocks that are present in nearly every tissue and organ system. Both the central and peripheral clocks can be reset by environmental cues, also known as Zeitgebers (German for 'timegivers'). The predominant Zeitgeber for the central clock is light, which is sensed by retina and signals directly to the SCN. The central clock entrains the peripheral clocks through neuronal and hormonal signals, body temperature and feeding-related cues, aligning all clocks with the external light/dark cycle. Circadian rhythms allow an organism to achieve temporal homeostasis with its environment at the molecular level by regulating gene expression to create a peak of protein expression once every 24 hours to control when a particular physiological process is most active with respect to the solar day. Transcription and translation of core clock components (CLOCK, NPAS2, ARNTL/BMAL1, ARNTL2/BMAL2, PER1, PER2, PER3, CRY1 and CRY2) plays a critical role in rhythm generation, whereas delays imposed by post-translational modifications (PTMs) are important for determining the period (tau) of the rhythms (tau refers to the period of a rhythm and is the length, in time, of one complete cycle). A diurnal rhythm is synchronized with the day/night cycle, while the ultradian and infradian rhythms have a period shorter and longer than 24 hours, respectively. Disruptions in the circadian rhythms contribute to the pathology of cardiovascular diseases, cancer, metabolic syndromes and aging. A transcription/translation feedback loop (TTFL) forms the core of the molecular circadian clock mechanism. Transcription factors, CLOCK or NPAS2 and ARNTL/BMAL1 or ARNTL2/BMAL2, form the positive limb of the feedback loop, act in the form of a heterodimer and activate the transcription of core clock genes and clock-controlled genes (involved in key metabolic processes), harboring E-box elements (5'-CACGTG-3') within

their promoters. The core clock genes: PER1/2/3 and CRY1/2 which are transcriptional repressors form the negative limb of the feedback loop and interact with the CLOCK NPAS2-ARNTL/BMAL1

ARNTL2/BMAL2 heterodimer inhibiting its activity and thereby negatively regulating their own expression. This heterodimer also activates nuclear receptors NR1D1/2 and RORA/B/G, which form a second feedback loop and which activate and repress ARNTL/BMAL1 transcription, respectively. ARNTL/BMAL1 positively regulates myogenesis and negatively regulates adipogenesis via the transcriptional control of the genes of the canonical Wnt signaling pathway. Plays a role in normal pancreatic beta-cell function; regulates glucose-stimulated insulin secretion via the regulation of antioxidant genes NFE2L2/NRF2 and its targets SESN2, PRDX3, CCLC and CCLM. Negatively regulates the mTORC1 signaling pathway; regulates the expression of MTOR and DEPTOR. Controls diurnal oscillations of Ly6C inflammatory monocytes; rhythmic recruitment of the PRC2 complex imparts diurnal variation to chemokine expression that is necessary to sustain Ly6C monocyte rhythms. Regulates the expression of HSD3B2, STAR, PTGS2, CYP11A1, CYP19A1 and LHCGR in the ovary and also the genes involved in hair growth. Plays an important role in adult hippocampal neurogenesis by regulating the timely entry of neural stem/progenitor cells (NSPCs) into the cell cycle and the number of cell divisions that take place prior to cell-cycle exit. Regulates the circadian expression of CIART and KLF11. The CLOCK-ARNTL/BMAL1 heterodimer regulates the circadian expression of SERPINE1/PAI1, VWF, B3, CCRN4L/NOC, NAMPT, DBP, MYOD1, PPARGC1A, PPARGC1B, SIRT1, GYS2, F7, NGFR, GNRHR, BHLHE40/DEC1, ATF4, MTA1, KLF10 and also genes implicated in glucose and lipid metabolism. Represses glucocorticoid receptor NR3C1/GR-induced transcriptional activity by reducing the association of NR3C1/GR to glucocorticoid response elements (GREs) via the acetylation of multiple lysine residues located in its hinge region. Promotes rhythmic chromatin opening, regulating the DNA accessibility of other transcription factors. The NPAS2-ARNTL/BMAL1 heterodimer positively regulates the expression of MAOA, F7 and LDHA and modulates the circadian rhythm of daytime contrast sensitivity by regulating the rhythmic expression of adenylate cyclase type 1 (ADCY1) in the retina.

Tissue specificity

Sequence similarities

Post-translational modifications

Hair follicles (at protein level). Highly expressed in the adult brain, skeletal muscle and heart.

Contains 1 bHLH (basic helix-loop-helix) domain.

Contains 1 PAC (PAS-associated C-terminal) domain.

Contains 2 PAS (PER-ARNT-SIM) domains.

Ubiquitinated, leading to its proteasomal degradation.

O-glycosylated; contains O-GlcNAc. O-glycosylation by OGT prevents protein degradation by inhibiting ubiquitination. It also stabilizes the CLOCK-ARNTL/BMAL1 heterodimer thereby increasing CLOCK-ARNTL/BMAL1-mediated transcription of genes in the negative loop of the circadian clock such as PER1/2/3 and CRY1/2.

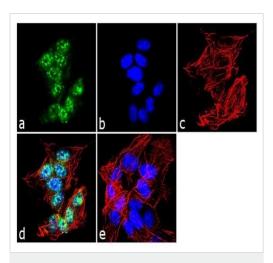
Acetylated on Lys-538 upon dimerization with CLOCK. Acetylation facilitates CRY1-mediated repression. Deacetylated by SIRT1, which may result in decreased protein stability. Phosphorylated upon dimerization with CLOCK. Phosphorylation enhances the transcriptional activity, alters the subcellular localization and decreases the stability of the CLOCK-ARNTL/BMAL1 heterodimer by promoting its degradation. Phosphorylation shows circadian variations in the liver with a peak between CT10 to CT14. Phosphorylation at Ser-90 by CK2 is essential for its nuclear localization, its interaction with CLOCK and controls CLOCK nuclear entry.

Sumoylated on Lys-259 upon dimerization with CLOCK. Predominantly conjugated to poly-SUMO2/3 rather than SUMO1 and the level of these conjugates undergo rhythmic variation, peaking at CT9-CT12. Sumoylation localizes it exclusively to the PML body and promotes its ubiquitination in the PML body, ubiquitin-dependent proteasomal degradation and the transcriptional activity of the CLOCK-ARNTL/BMAL1 heterodimer.

Cellular localization

Nucleus. Cytoplasm. Nucleus, PML body. Shuttles between the nucleus and the cytoplasm and this nucleocytoplasmic shuttling is essential for the nuclear accumulation of CLOCK, target gene transcription and the degradation of the CLOCK-ARNTL/BMAL1 heterodimer. The sumoylated form localizes in the PML body. Sequestered to the cytoplasm in the presence of ID2.

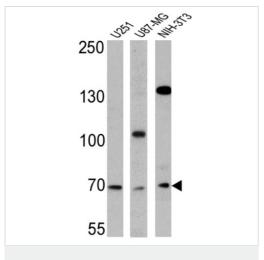
Images



Immunocytochemistry - Anti-BMAL1 antibody (ab3350)

Immunocytochemistry analysis of 4% paraformaldehyde-fixed SH-SY5Y cells permeabilised with 0.1% Triton™ X-100 (10 mins) and blocked with 1% BSA (1 hour).

a) BMAL1 is stained with ab3350 at 2µg/ml concentration and Goat anti-Rabbit lgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate in 1/2000 dilution (green). b) Nuclear staining with DAPI and SlowFade® Gold Antifade Mountant (blue). c) Factin stainined with a 1/3000 dilution Alexa Fluor® 555 Rhodamine Phalloidin (red). d) merged image. e) control with no primary antibody.



Western blot - Anti-BMAL1 antibody (ab3350)

All lanes: Anti-BMAL1 antibody (ab3350) at 1/500 dilution

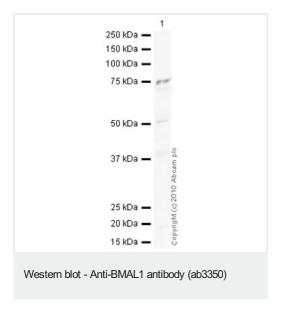
Lane 1 : U251 cell lysate

Lane 2 : U87-MG cell lysate

Lane 3 : NIH-3T3 cell lysate

Lysates/proteins at 25 µg per lane.

Predicted band size: 69.4 kDa **Observed band size:** 69 kDa



Anti-BMAL1 antibody (ab3350) at 1 μ g/ml + HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 μ g

Secondary

Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Predicted band size: 69.4 kDa **Observed band size:** 75 kDa

Additional bands at: 51 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 3 minutes

The 75 kDa band observed is comparable to the molecular weight seen with other commercially available antibodies to BMAL1.

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