# abcam

# Product datasheet

# Anti-BMAL1 antibody [EPR20906-14] - BSA and Azide free ab269960





RabMAb

# 3 Images

#### Overview

Product name Anti-BMAL1 antibody [EPR20906-14] - BSA and Azide free

**Description** Rabbit monoclonal [EPR20906-14] to BMAL1 - BSA and Azide free

Host species Rabbit

**Tested applications** Suitable for: WB, IP

Unsuitable for: Flow Cyt,ICC/IF,IHC-Fr or IHC-P

Species reactivity Reacts with: Mouse, Rat

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Rat liver and brain tissue lysate; mouse testis lysate; mouse wild-type liver nuclear fraction.

IP: Mouse testis lysate.

**General notes** ab269960 is the carrier-free version of <u>ab235577</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR20906-14

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab269960 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		Use at an assay dependent concentration. Detects a band of approximately 75 kDa (predicted molecular weight: 68 kDa).
IP		Use at an assay dependent concentration.

**Application notes** 

Is unsuitable for Flow Cyt,ICC/IF,IHC-Fr or IHC-P.

#### **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.

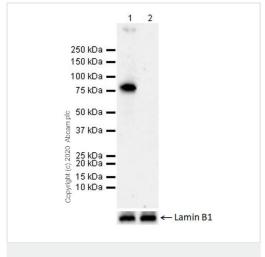
Phosphorylated upon dimerization with CLOCK. Phosphorylation enhances the transcriptional activity, alters the subcellular localization and decreases the stability of the CLOCK-ARNTL/BMAL1 heterodimer by promoting its degradation. Phosphorylation shows circadian variations in the liver with a peak between 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**



Western blot - Anti-BMAL1 antibody [EPR20906-14]
- BSA and Azide free (ab269960)

**All lanes :** Anti-BMAL1 antibody [EPR20906-14] (ab235577) at 1/1000 dilution

Lane 1: Wild type mouse liver nuclear lysate

Lane 2: Bmal1 knockout mouse liver nuclear lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

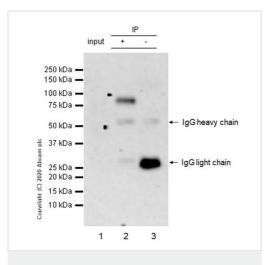
Predicted band size: 68 kDa
Observed band size: 75 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Lysates were kindly provided by Dr Zhang Erquan.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab235577).



Immunoprecipitation - Anti-BMAL1 antibody

[EPR20906-14] - BSA and Azide free (ab269960)

BMAL1 was immunoprecipitated from 0.35 mg Mouse testis tissue lysate 10 ug with <u>ab235577</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab235577</u> at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: Mouse testis tissue lysate 10 ug

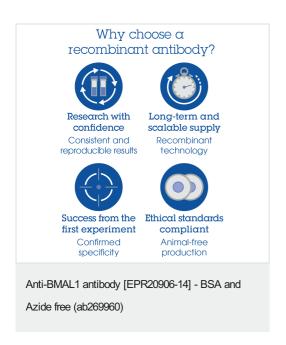
Lane 2: ab235577 IP in Mouse testis tissue lysate

Lane 3: Rabbit monoclonal  $\lg G$  ( $\underline{ab172730}$ ) instead of  $\underline{ab235577}$  in mouse testis tissue lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab235577).



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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