# abcam

# Product datasheet

# Anti-p27 KIP 1 antibody [EPFHCR16] - BSA and Azide free ab247597





RabMAb

# 7 Images

#### Overview

**Product name** Anti-p27 KIP 1 antibody [EPFHCR16] - BSA and Azide free

Rabbit monoclonal [EPFHCR16] to p27 KIP 1 - BSA and Azide free **Description** 

**Host species** Rabbit

**Tested applications** Suitable for: IHC-P, WB

Unsuitable for: Flow Cyt,ICC/IF or IP

Species reactivity Reacts with: Mouse, Rat

**Immunogen** Recombinant fragment within Mouse p27 KIP 1. The exact sequence is proprietary.

**General notes** ab247597 is the carrier-free version of ab92741.

> Our carrier-free 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 cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits 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**.

Human: We have preliminary internal testing data to indicate this antibody may not react with this

## **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

ClonalityMonoclonalClone numberEPFHCR16

**Isotype** IgG

# **Applications**

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab247597 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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 27 kDa (predicted molecular weight: 22 kDa).

**Application notes** Is unsuitable for Flow Cyt,ICC/IF or IP.

#### **Target**

Function Important regulator of cell cycle progression. Involved in G1 arrest. Potent inhibitor of cyclin E- and cyclin A-CDK2 complexes. Forms a complex with cyclin type D-CDK4 complexes and is involved in the assembly, stability, and modulation of CCND1-CDK4 complex activation. Acts either as an

inhibitor or an activator of cyclin type D-CDK4 complexes depending on its phosphorylation state

and/or stoichometry.

**Tissue specificity** Expressed in all tissues tested. Highest levels in skeletal muscle, lowest in liver and kidney.

**Involvement in disease** Defects in CDKN1B are the cause of multiple endocrine neoplasia type 4 (MEN4) [MIM:610755].

Multiple endocrine neoplasia (MEN) syndromes are inherited cancer syndromes of the thyroid.

MEN4 is a MEN-like syndrome with a phenotypic overlap of both MEN1 and MEN2.

**Sequence similarities**Belongs to the CDI family.

**Domain** A peptide sequence containing only AA 28-79 retains substantial Kip1 cyclin A/CDK2 inhibitory

activity.

**Post-translational** Phosphorylated; phosphorylation occurs on serine, threonine and tyrosine residues.

#### modifications

Phosphorylation on Ser-10 is the major site of phosphorylation in resting cells, takes place at the G(0)-G(1) phase and leads to protein stability. Phosphorylation on other sites is greatly enhanced by mitogens, growth factors, cMYC and in certain cancer cell lines. The phosphorylated form found in the cytoplasm is inactivate. Phosphorylation on Thr-198 is required for interaction with 14-3-3 proteins. Phosphorylation on Thr-187, by CDK2 leads to protein ubiquitination and proteasomal degradation. Tyrosine phosphorylation promotes this process. Phosphorylation by PKB/AKT1 can be suppressed by LY294002, an inhibitor of the catalytic subunit of Pl3K. Phosphorylation on Tyr-88 and Tyr-89 has no effect on binding CDK2, but is required for binding CDK4. Dephosphorylated on tyrosine residues by G-CSF.

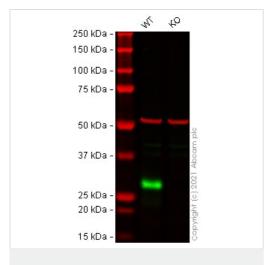
Ubiquitinated; in the cytoplasm by the KPC complex (composed of RNF123/KPC1 and UBAC1/KPC2) and, in the nucleus, by SCF(SKP2). The latter requires prior phosphorylation on Thr-187. Ubiquitinated; by a TRIM21-containing SCF(SKP2)-like complex; leads to its degradation.

Subject to degradation in the lysosome. Interaction with SNX6 promotes lysosomal degradation.

**Cellular localization** 

Nucleus. Cytoplasm. Endosome. Nuclear and cytoplasmic in quiescent cells. AKT-or RSK-mediated phosphorylation on Thr-198, binds 14-3-3, translocates to the cytoplasm and promotes cell cycle progression. Mitogen-activated UHMK1 phosphorylation on Ser-10 also results in translocation to the cytoplasm and cell cycle progression. Phosphorylation on Ser-10 facilitates nuclear export. Translocates to the nucleus on phosphorylation of Tyr-88 and Tyr-89. Colocalizes at the endosome with SNX6 and this leads to lysosomal degradation.

#### **Images**



Western blot - Anti-p27 KIP 1 antibody [EPFHCR16] - BSA and Azide free (ab247597)

**All lanes :** Anti-p27 KIP 1 antibody [EPFHCR16] (ab92741) at 1/1000 dilution

Lane 1: Wild-type RAW 264.7 cell lysate

Lane 2: CDKN1B knockout RAW 264.7 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 22 kDa Observed band size: 28 kDa

False colour image of Western blot: Anti-p27 KIP 1 antibody [EPFHCR16] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab92741 was shown to bind specifically to p27 KIP 1. A band was observed at 28 kDa in wild-type RAW 264.7 cell lysates with no signal observed at this size in CDKN1B knockout cell line ab281619 (knockout cell lysate ab282970). To generate this image, wild-type and CDKN1B

knockout RAW 264.7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween<sup>®</sup> 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit lgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (ab216776) at 1/20000 dilution.

kDa
1 2 3

10075503725201510-

Western blot - Anti-p27 KIP 1 antibody [EPFHCR16]

- BSA and Azide free (ab247597)

**All lanes :** Anti-p27 KIP 1 antibody [EPFHCR16] (<u>ab92741</u>) at 1/1000 dilution

Lane 1: NIH/3T3 cell lysate

Lane 2: C6 cell lysate

Lane 3: Neuro-2a cell lysate

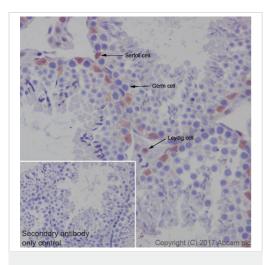
Lysates/proteins at 10 µg per lane.

### Secondary

All lanes: Goat anti-rabbit HRP-conjugated at 1/2000 dilution

**Predicted band size:** 22 kDa **Observed band size:** 27 kDa

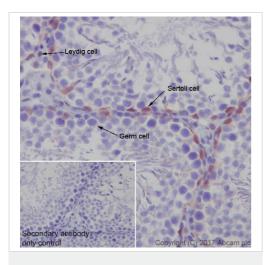
This data was developed using <u>ab92741</u>, the same antibody clone in a different buffer formulation.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p27 KIP 1 antibody

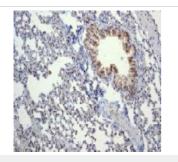
[EPFHCR16] - BSA and Azide free (ab247597)

This data was developed using <u>ab92741</u>, the same antibody clone in a different buffer formulation.lmmunohistochemical analysis of paraffin-embedded mouse testis tissue labeling p27 KIP 1 with <u>ab92741</u> at 1/5000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining in Leydig and Sertoli cells and Leydig cells of mouse testis is observed (PMID: 10098522). Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody. Heat mediated antigen retrieval was performed using Universal HIER antigen retrieval reagent (10X) (<u>ab208572</u>).



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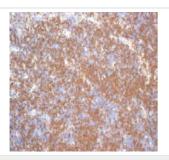
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This data was developed using <u>ab92741</u>, the same antibody clone in a different buffer formulation.<u>ab92741</u> at 1/100 dilution staining p27 KIP in Mouse lung by Immunohistochemistry, Paraffinembedded tissue. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p27 KIP 1 antibody

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This data was developed using <u>ab92741</u>, the same antibody clone in a different buffer formulation.<u>ab92741</u> at 1/100 dilution staining p27 KIP in Mouse thymus by Immunohistochemistry, Paraffinembedded tissue. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



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