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Product datasheet

Anti-p27 KIP 1 (phospho S10) antibody [EP233(2)Y] ab62364

Recombinant RabMAb

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Overview

Product name	Anti-p27 KIP 1 (phospho S10) antibody [EP233(2)Y]
Description	Rabbit monoclonal [EP233(2)Y] to p27 KIP 1 (phospho S10)
Host species	Rabbit
Specificity	This antibody detects p27 KIP 1 phosphorylated at Serine 10. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** Dot blot, WB, IP, IHC-P **Unsuitable for:** Flow Cyt or ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human, Monkey
Immunogen	Synthetic peptide within Human p27 KIP 1 aa 1-100 (phospho S10) (Cysteine residue). The exact sequence is proprietary. Database link: **P46527**
Positive control	IP: MCF7 cell lysate; IHC: Human colon cancer; WB: HeLa, NIH/3T3, and C6 whole cell lysates.
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP233(2)Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab62364 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
Dot blot		1/1000.
WB	★★★★☆ (1)	1/500 - 1/20000. Detects a band of approximately 27 kDa (predicted molecular weight: 22 kDa).
IP		1/50.
IHC-P	★★★★★ (4)	1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified use at 1/200 - 1/1500** The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.

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

Target

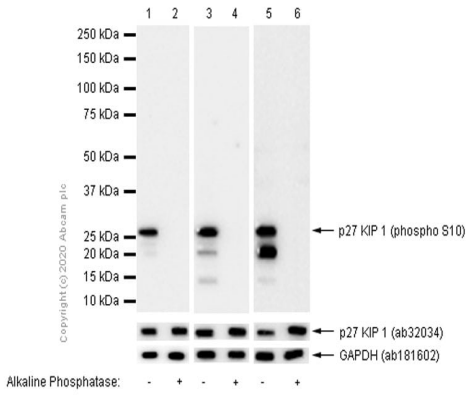
Function	Important regulator of cell cycle progression. Involved in G1 arrest. Potent inhibitor of cyclin E- and cyclin A-CDK2 complexes. Forms a complex with cyclin type D-CDK4 complexes and is involved in the assembly, stability, and modulation of CCND1-CDK4 complex activation. Acts either as an inhibitor 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 modifications	Phosphorylated; phosphorylation occurs on serine, threonine and tyrosine residues. 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 PI3K. 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 (phospho S10) antibody [EP233(2)Y] (ab62364)

All lanes : Anti-p27 KIP 1 (phospho S10) antibody [EP233(2)Y] (ab62364) at 1/1000 dilution (Purified)

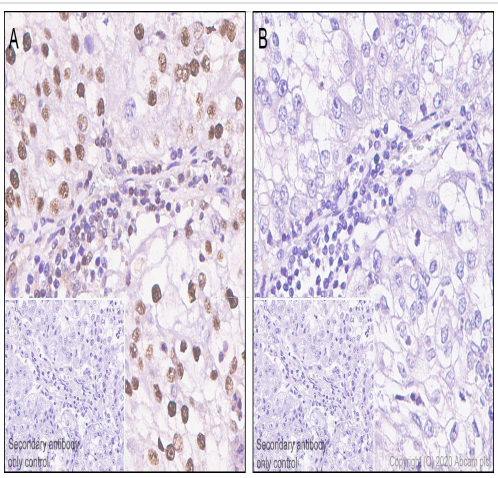
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate, membrane treated with Alkaline Phosphatase for 1 hour
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 4 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate, membrane treated with Alkaline Phosphatase for 1 hour
Lane 5 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 6 : C6 (Rat glial tumor glial cell) whole cell lysate, membrane treated with Alkaline Phosphatase for 1 hour

Lysates/proteins at 15 µg per lane.

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

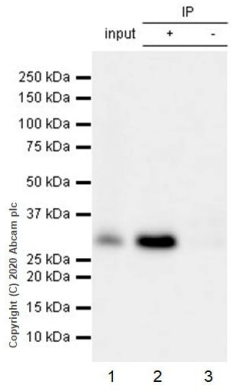
Predicted band size: 22 kDa
Observed band size: 27 kDa

Blocking buffer: 5% NFDM/TBST



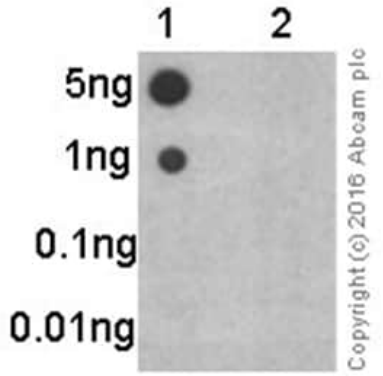
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 (phospho S10) antibody [EP233(2)Y] (ab62364)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon cancer tissue sections labeling p27 KIP 1 with Purified ab62364 at 1:100 dilution (4.19 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunoprecipitation - Anti-p27 KIP 1 (phospho S10) antibody [EP233(2)Y] (ab62364)

Purified ab62364 at 1/50 dilution (2µg) immunoprecipitating p27 KIP 1 in MCF7 whole cell lysate.
Lane 1 (input): MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab62364 + MCF7 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab62364 in MCF7 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 27 kDa



Dot Blot - Anti-p27 KIP 1 (phospho S10) antibody [EP233(2)Y] (ab62364)

Dot blot analysis of p27 KIP 1 (pS10) phospho peptide (Lane 1) and p27 KIP 1 non-phospho peptide (Lane 2) using ab62364 at 1/1000 dilution followed by Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution.

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

Exposure time: 3 minutes.

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