abcam

Product datasheet

HRP Anti-p27 KIP 1 antibody [Y236] ab194235

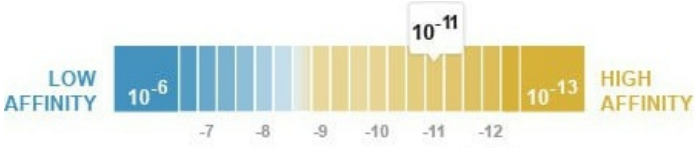
KO VALIDATED Recombinant RabMAb

4 Images

Overview

Product name	HRP Anti-p27 KIP 1 antibody [Y236]
Description	HRP Rabbit monoclonal [Y236] to p27 KIP 1
Host species	Rabbit
Conjugation	HRP
Tested applications	**Suitable for:** WB, IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: FFPE human colon adenocarcinoma.
General notes	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. Avoid freeze / thaw cycle. Store In the Dark.
Dissociation constant (K_D)	$K_D = 2.10 \times 10^{-11}$ M  **Learn more about K_D**
Storage buffer	pH: 7.40 Preservative: 0.1% Proclin 300 Solution Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	Y236
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab194235 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. Predicted molecular weight: 22 kDa.
IHC-P		1/70 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

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 - HRP Anti-p27 KIP 1 antibody [Y236] (ab194235)

All lanes : HRP Anti-p27 KIP 1 antibody [Y236] (ab194235) at 1/5000 dilution

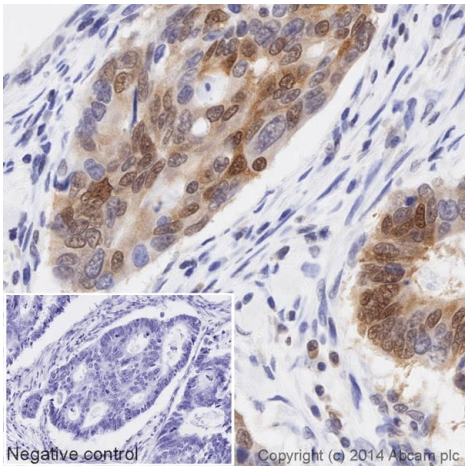
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : CDKN1B (p27 KIP 1) knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 22 kDa

Exposure time: 2 minutes

ab194235 was shown to recognize p27 KIP 1 in wild-type HAP1 cells as signal was lost at the expected MW in CDKN1B (p27 KIP 1) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and CDKN1B (p27 KIP 1) knockout samples were subjected to SDS-PAGE. Ab194235 and **ab184095** (Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control (Alexa Fluor® 680) loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/1000 dilution respectively. The loading control was imaged using the Licor Odyssey CLx prior to blots being developed with ECL technique.



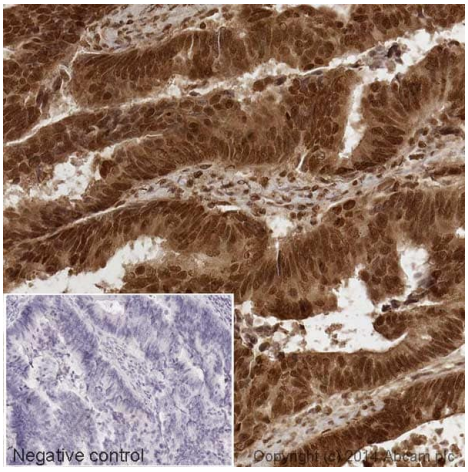
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - HRP Anti-p27 KIP 1 antibody [Y236] (ab194235)

IHC image of p27 KIP 1 staining in a section of formalin-fixed paraffin-embedded human colon adenocarcinoma*, performed on a Leica BOND. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab194235 at 1/70 dilution, for 15 mins at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - HRP Anti-p27 KIP 1 antibody [Y236] (ab194235)

IHC image of p27 KIP 1 staining in a section of formalin-fixed paraffin-embedded human colon adenocarcinoma*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins, and incubated overnight at +4°C with ab194235 at 1µg/ml. DAB was used as the chromogen (**ab103723**), diluted 1/100 and incubated for 10min at room temperature. The section was counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and manual) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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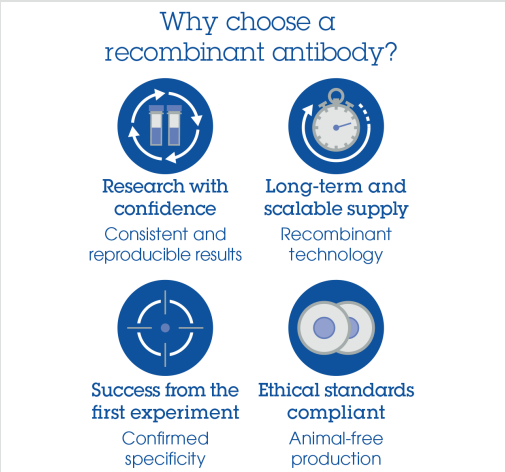

HRP Anti-p27 KIP 1 antibody [Y236] (ab194235)

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