abcam

Product datasheet

Anti-p27 KIP 1 antibody [Y236] ab32034

敲除验证 重组 RabMAb

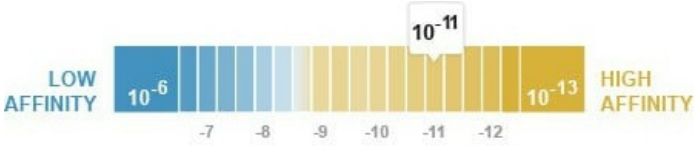
18 Abreviews 186 References 23 图像

概述

产品名称	Anti-p27 KIP 1抗体[Y236]
描述	兔单克隆抗体[Y236] to p27 KIP 1
宿主	Rabbit
特异性	This antibody recognises p27(Kip1). The rat recommendation is based on the WB results. We do not guarantee IHC-P for rat.
经测试应用	**适用于:** IHC-P, Flow Cyt (Intra), WB, ICC/IF, IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide corresponding to Human p27 KIP 1 aa 150-250 (C terminal).
阳性对照	WB: HAP1, HeLa, MCF-7 cell lysate IHC: Ovarian carcinoma, human breast carcinoma, Colonic adenocarcinoma, Stomach adenocarcinoma, human stomach and Glioma tissue. ICC/IF: MCF-7 and HeLa cells Flow Cyt (intra): HAP1-WT, NIH/3T3 cells
常规说明	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**.

性能

形式	Liquid
存放说明	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.
解离常数(K_D)	K_D = 2.10 x 10 $^{-11}$ M



Learn more about K_D

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	Y236
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab32034于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IHC-P	★★★★☆ (3)	1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. The rat recommendation is based on the WB results. We do not guarantee IHC-P for rat.
Flow Cyt (Intra)		1/50. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/20 - 1/40.
WB	★★★★★ (8)	1/5000. Detects a band of approximately 27 kDa (predicted molecular weight: 22 kDa). **For unpurified use at 1:1000.**
ICC/IF	★★★★★ (2)	1/100 - 1/500.
IP	★★★★☆ (1)	1/30. **For unpurified use at 1:50.**

靶标

功能	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.
组织特异性	Expressed in all tissues tested. Highest levels in skeletal muscle, lowest in liver and kidney.
疾病相关	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.
序列相似性	Belongs to the CDI family.
结构域	A peptide sequence containing only AA 28-79 retains substantial Kip1 cyclin A/CDK2 inhibitory

activity.

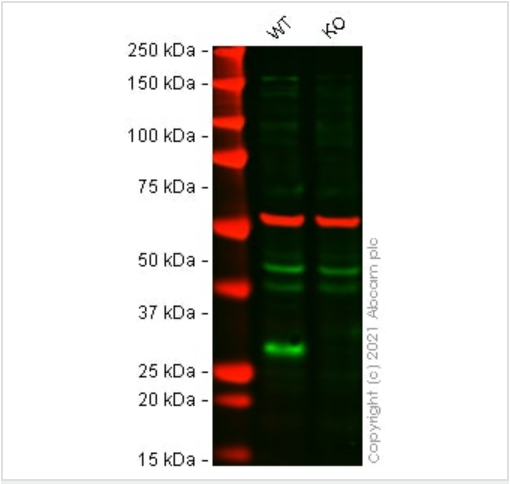
翻译后修饰

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.

细胞定位

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.

图片



Western blot - Anti-p27 KIP 1 antibody [Y236] (ab32034)

All lanes : Anti-p27 KIP 1 antibody [Y236] (ab32034) at 1/5000 dilution

Lane 1 : Wild-type RAW 264.7 cell lysate
Lane 2 : CDKN1B knockout RAW 264.7 cell lysate

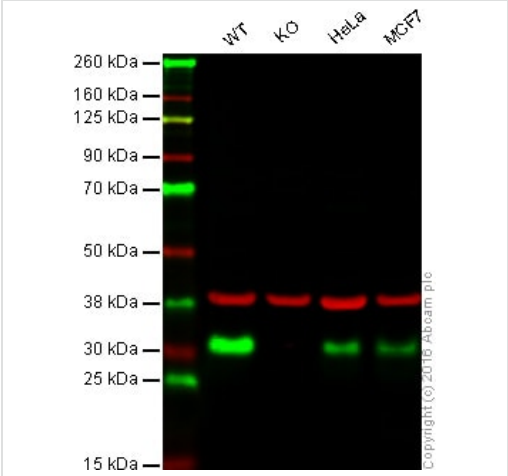
Lysates/proteins at 20 µg per lane.

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 [Y236] staining at 1/5000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32034 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® 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 IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.

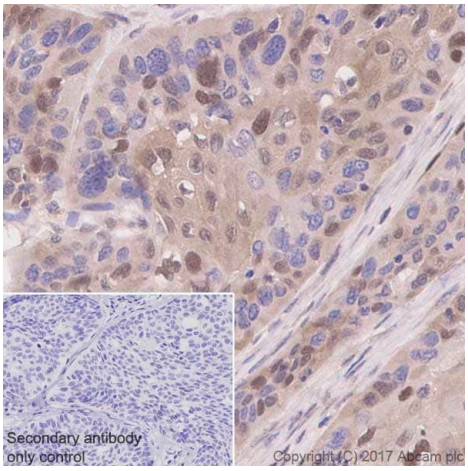


Western blot - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: CDKN1B knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: MCF7 whole cell lysate (20 µg)

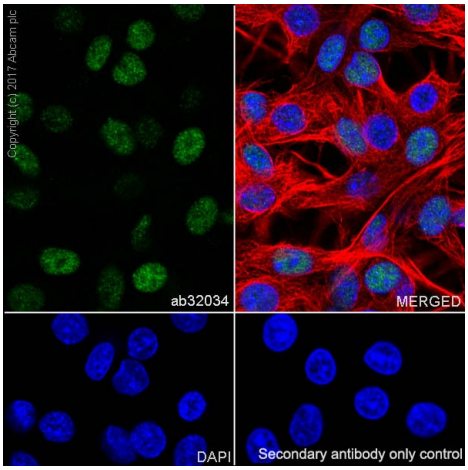
Lanes 1 - 4: Merged signal (red and green). Green - ab32034 observed at 30 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

Unpurified ab32034 was shown to specifically react with CDKN1B in wild-type HAP1 cells. No band was observed when CDKN1B knockout samples were used. Wild-type and CDKN1B knockout samples were subjected to SDS-PAGE. ab32034 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 and 1/10000 respectively. Blots were developed with 800CW Goat anti Rabbit and 680CW Goat anti Mouse secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



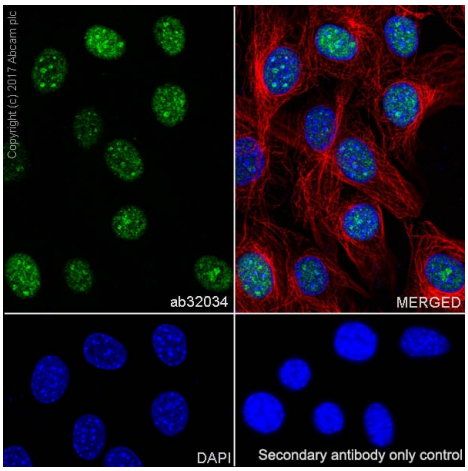
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human cervical carcinoma tissue sections labeling p27 KIP 1 with Purified ab32034 at 1:50 dilution (10.4 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Immunocytochemistry/ Immunofluorescence - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Immunocytochemistry/ Immunofluorescence analysis of RAW264.7(Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 7 h and 1µg/ml BFA for the last 3h cells labeling p27 KIP 1 with purified ab32034 at 1/100 dilution(5.22 µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 dilution (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunocytochemistry/ Immunofluorescence - Anti-p27 KIP 1 antibody [Y236] (ab32034)

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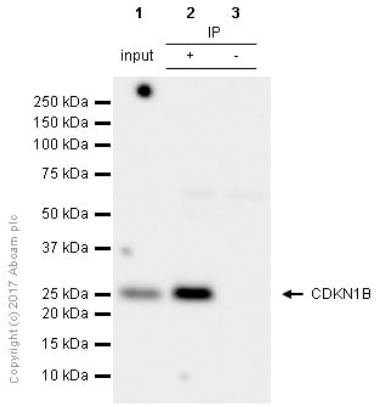
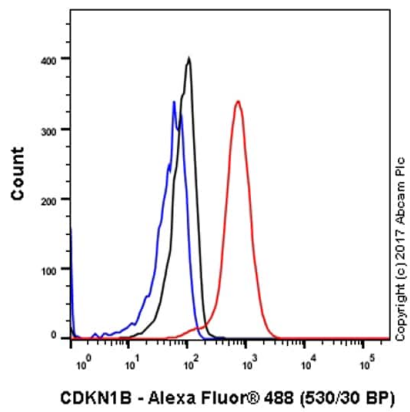


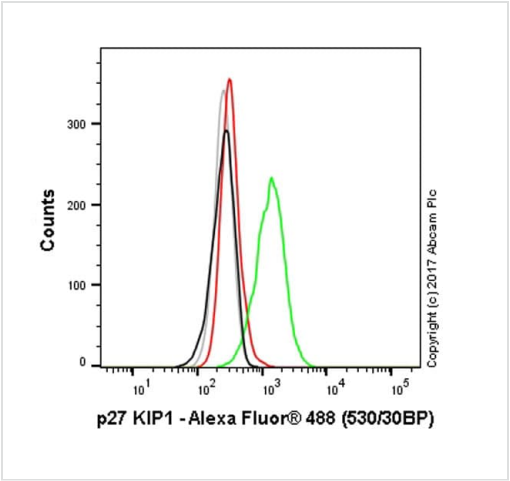
Immunoprecipitation - Anti-p27 KIP 1 antibody [Y236] (ab32034)

ab32034 (purified) at 1/30 dilution (20 µg/mL) immunoprecipitating p27 KIP 1 in C6 whole cell lysate.
Lane 1 (input): C6(Rat glial tumor glial cell) whole cell lysate 10 µg
Lane 2 (+): ab32034 & C6 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32034 in C6 whole cell lysate
For western blotting, ab32034 at 1/500 dilution (1.044 µg/mL) and VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM /TBST .



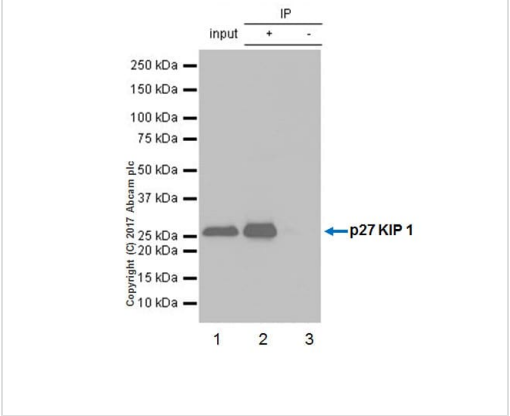
Flow Cytometry (Intracellular) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Intracellular Flow Cytometry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labelling p27 KIP 1 with ab32034 at 1/500 dilution (5.22 µg/ml) (Red). Cells were fixed with 4% paraformaldehyde . Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) (Black). Unlabeled control - Unlabelled cells (Blue).



Flow Cytometry (Intracellular) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-CDKN1B knockout cells (red line) stained with ab32034. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab32034, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-CDKN1B knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 4%PFA (10 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

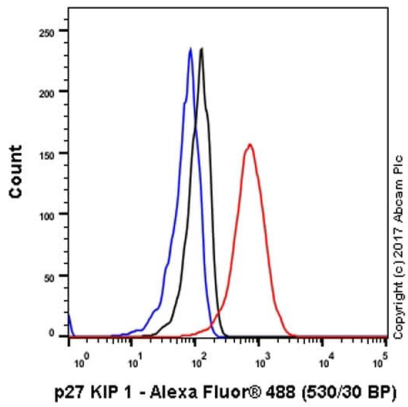


Immunoprecipitation - Anti-p27 KIP 1 antibody [Y236] (ab32034)

ab32034 (purified) at 1/20 dilution (2µg) immunoprecipitating p27 KIP 1 in MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate.

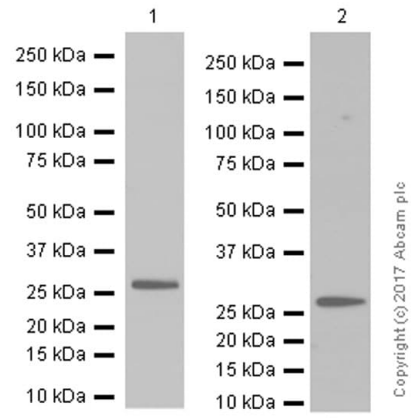
Lane 1 (input): MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab32034 & MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32034 in MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling p27 KIP 1 with purified ab32034 at 1/50 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-p27 KIP 1 antibody [Y236] (ab32034)

All lanes : Anti-p27 KIP 1 antibody [Y236] (ab32034) at 1/5000 dilution (purified)

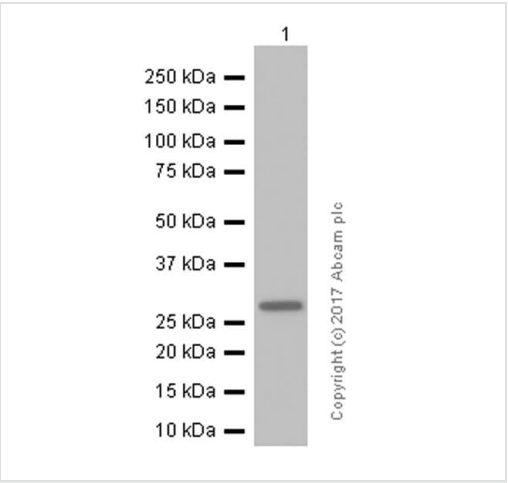
Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : C6 (Rat glial tumor glial cell) whole cell lysates

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

Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-p27 KIP 1 antibody [Y236] (ab32034)

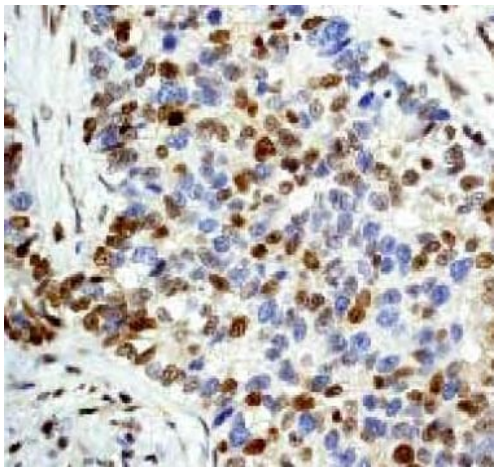
Anti-p27 KIP 1 antibody [Y236] (ab32034) at 1/5000 dilution (purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 15 µg

Secondary

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

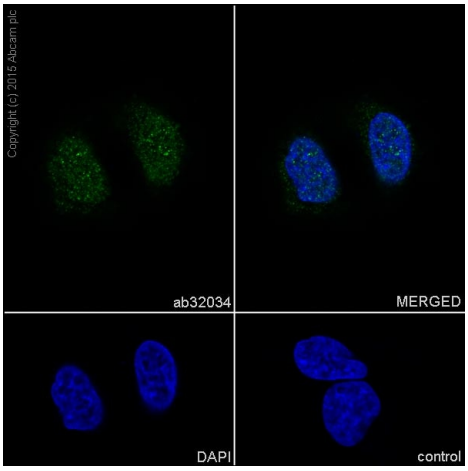
Predicted band size: 22 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

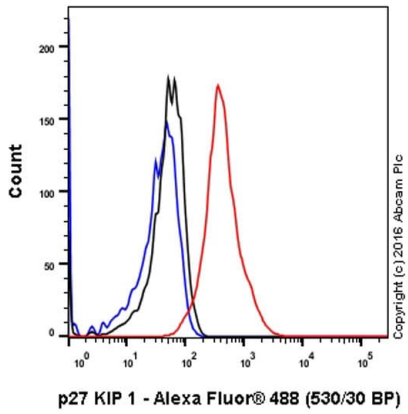
Immunohistochemical analysis of paraffin-embedded human breast carcinoma. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) cells labelling p27 KIP 1 (green) with purified ab32034 at 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Nuclei were counterstained with DAPI (blue).

Secondary Only Control: PBS was used instead of the primary antibody as the negative control.

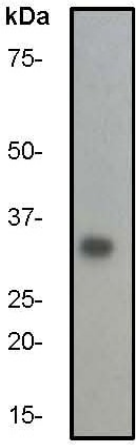


Flow Cytometry (Intracellular) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Unpurified ab32034 staining p27 KIP 1in the human cell line MCF-7 (human breast carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde, permeabilized with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

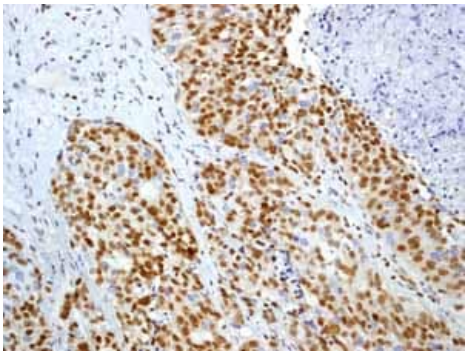
Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-p27 KIP 1 antibody [Y236] (ab32034)

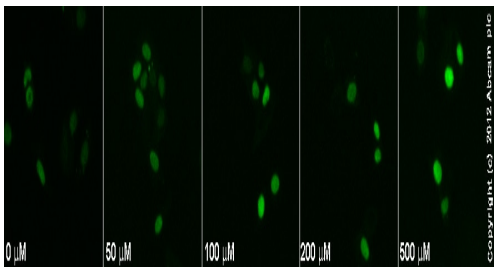
Anti-p27 KIP 1 antibody [Y236] (ab32034) at 1/1000 dilution (unpurified) + MCF-7 cell lysate

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

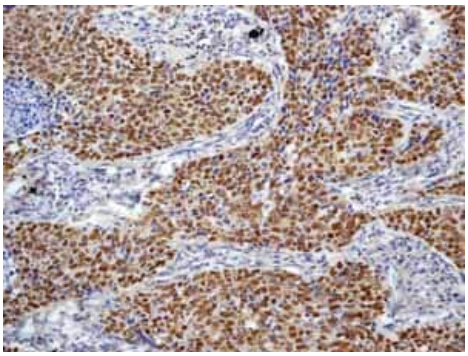
Unpurified ab32034 showing positive staining in Ovarian carcinoma tissue. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-p27 KIP 1 antibody [Y236] (ab32034)

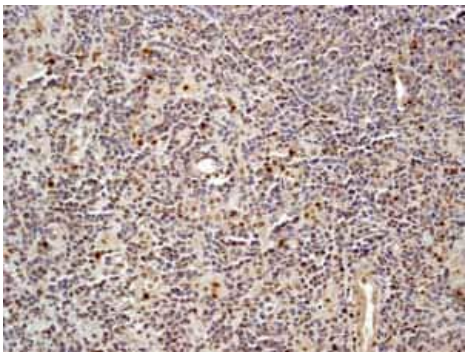
Unpurified ab32034 staining p27 KIP1 in MCF7 cells treated with NS 398 (**ab120295**), by ICC/IF. Increase in p27 KIP1 expression correlates with increased concentration of NS 398, as described in literature.

The cells were incubated at 37°C for 6h in media containing different concentrations of **ab120295** (NS 398) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab32034 (1/100 dilution) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody.



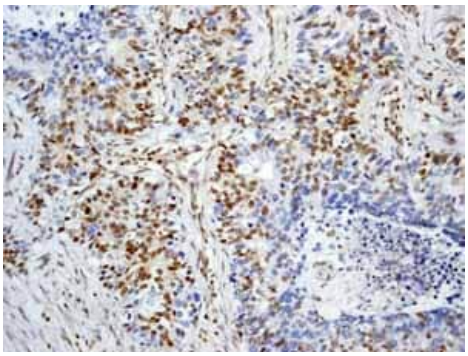
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Unpurified ab32034 showing positive staining in Colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0).



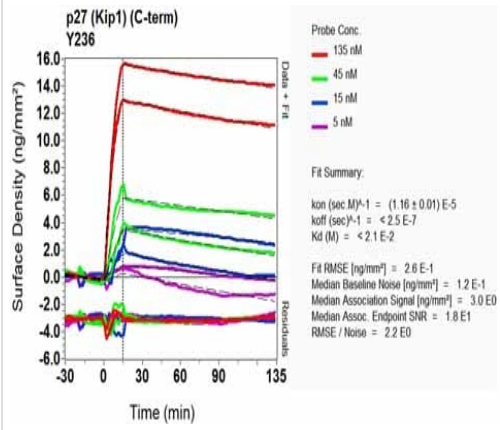
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Unpurified ab32034 showing positive staining in Glioma tissue. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Unpurified ab32034 showing positive staining in Stomach adenocarcinoma tissue. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0).



OI-RD Scanning - Anti-p27 KIP 1 antibody [Y236] (ab32034)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-p27 KIP 1 antibody [Y236] (ab32034)

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