abcam

Product datasheet

Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free ab206927

KO VALIDATED Recombinant RabMAb

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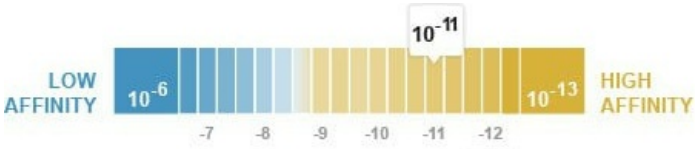
Overview

Product name	Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free
Description	Rabbit monoclonal [Y236] to p27 KIP 1 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody recognises p27(Kip1). The rat recommendation is based on the WB results. We do not guarantee IHC-P for rat.
Tested applications	**Suitable for:** Flow Cyt (Intra), IHC-P, WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	MCF-7 cell lysate, PC-12 cells, human breast carcinoma
General notes	ab206927 is the carrier-free version of **ab32034**. 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 cell-based 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® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® 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.
Dissociation constant (K_D)	$K_D = 2.10 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y236
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab206927 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

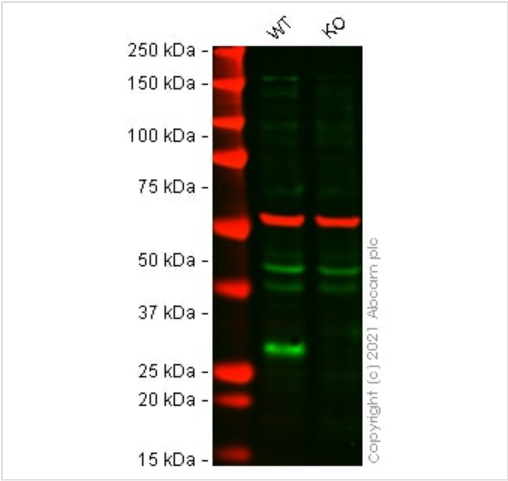
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 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).
ICC/IF	★★★★☆ (2)	Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

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 antibody [Y236] - BSA and Azide free (ab206927)

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

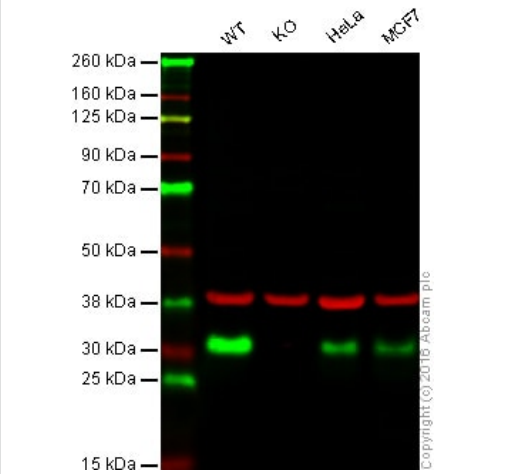
Lane 1 : Wild-type RAW 264.7 cell lysate
Lane 2 : CDKN1B knockout RAW 264.7 cell lysate

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.



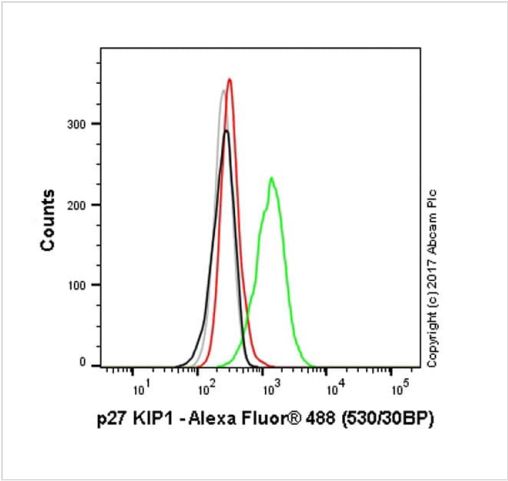
Western blot - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

This WB data was generated using the same anti-p27 KIP 1 antibody clone, Y236, in a different buffer formulation (cat# **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.

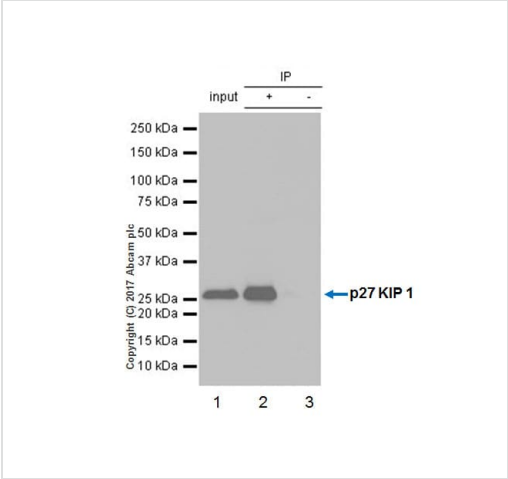
Unpurified **ab32034** was shown to specifically react with CDKN1B in wild-type HAP1 cells. No band was observed when CDKN1B knockout samples were used. Wild-type and CDKN1B knockout samples were subjected to SDS-PAGE. **ab32034** and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight 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.



Flow Cytometry (Intracellular) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



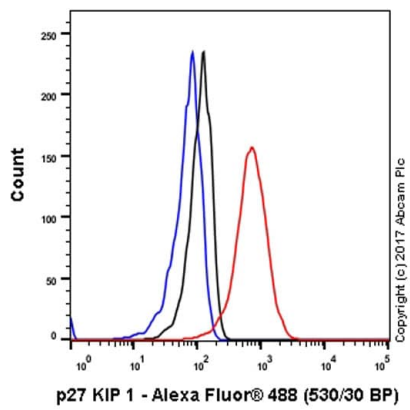
Immunoprecipitation - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

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.

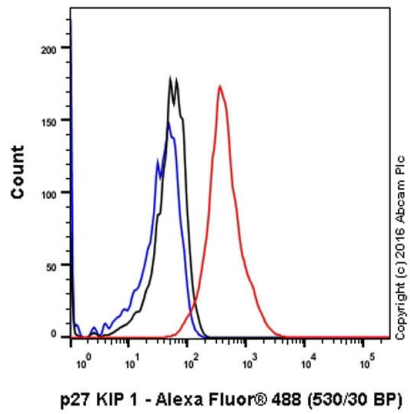
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



Flow Cytometry (Intracellular) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling p27 KIP 1 with purified **ab32034** at 1/50 dilution (10 µg/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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



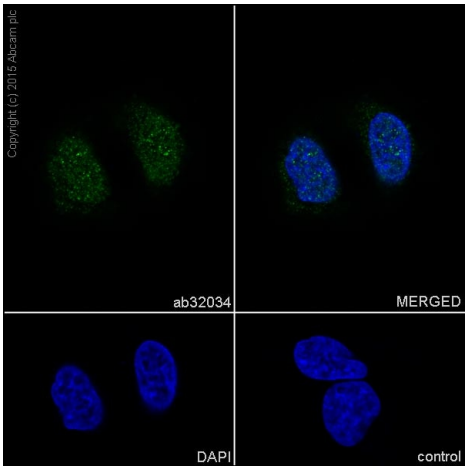
Flow Cytometry (Intracellular) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

Unpurified **ab32034** staining p27 KIP 1 in 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)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).

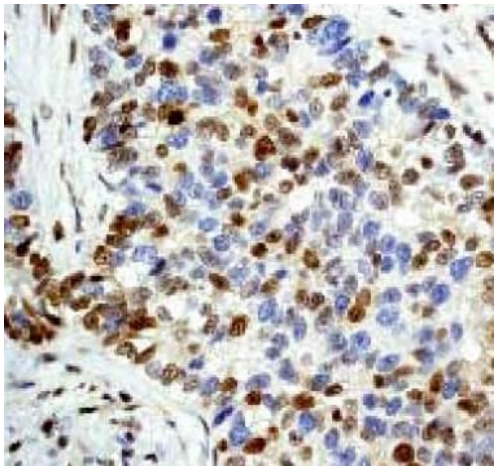


Immunocytochemistry/ Immunofluorescence - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

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).

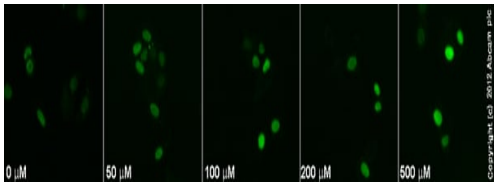
Secondary Only Control: PBS was used instead of the primary antibody as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

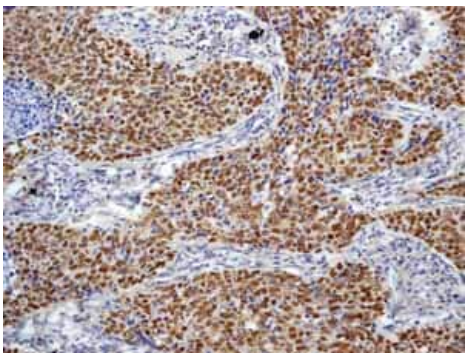
Immunohistochemical analysis of paraffin-embedded human breast carcinoma
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



Immunocytochemistry/ Immunofluorescence - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

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.

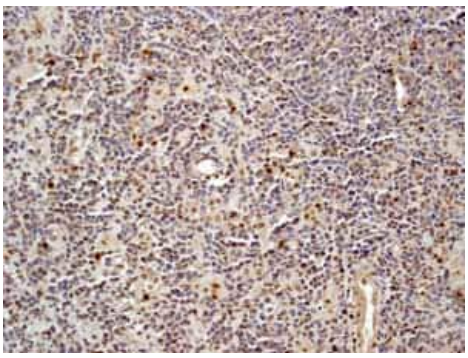
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

Unpurified **ab32034** showing positive staining in Colonic adenocarcinoma tissue.

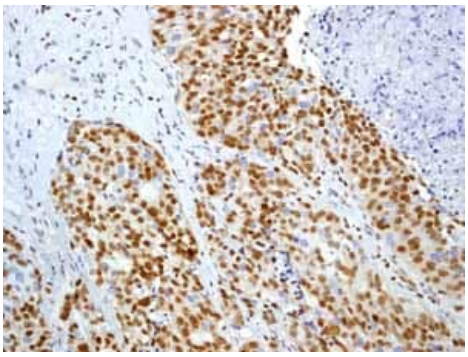
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

Unpurified **ab32034** showing positive staining in Glioma tissue.

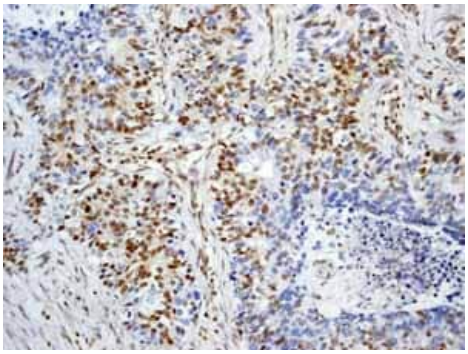
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

Unpurified **ab32034** showing positive staining in Ovarian carcinoma tissue.

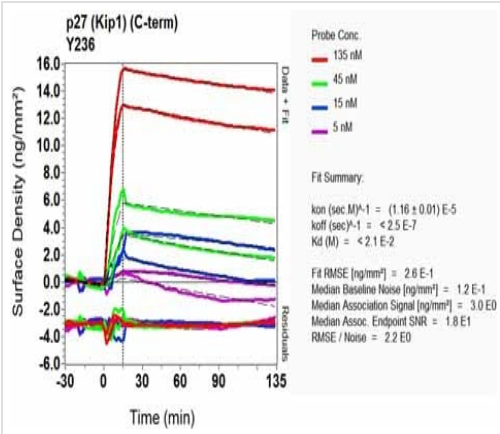
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

Unpurified **ab32034** showing positive staining in Stomach adenocarcinoma tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



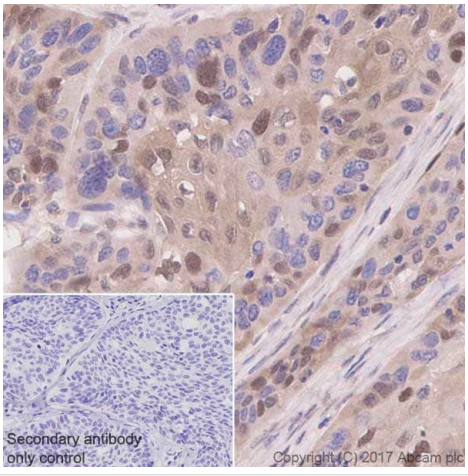
OI-RD Scanning - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

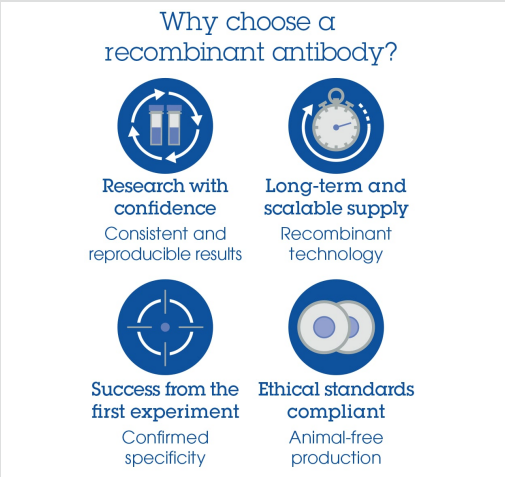
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32034**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

This IHC data was generated using the same anti-p27 KIP 1 antibody clone, Y236, in a different buffer formulation (cat# **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.



Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

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