abcam

ab206927

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

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	Y236
Purification technique	Affinity purification Protein A
Specificity	This antibody recognises p27(Kip1).
Dissociation constant	2.1 x 10^{-11} M
Concentration	1.029 - 1.052 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Rat

Dilution info	-
Notes	-

Expected

Species	Human, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Rat, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information CDKN1B

Function	Important regulator of cell cycle progression. Inhibits the kinase activity of CDK2 bound to cyclin A, but has little inhibitory activity on CDK2 bound to SPDYA (PubMed:28666995). 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.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab206927 is the carrier-free version of ab32034.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The protein known as p27 KIP1 also referred to as p27 CDKN1B or KIP1 is a member of the kinase inhibitory protein family. It plays an important role in cell cycle regulation by inhibiting cyclin-dependent kinases (CDKs). p27 KIP1 weighs approximately 27 kDa and can be found in various tissues where it regulates cell proliferation. The protein functions as a suppressor by binding to cyclin-CDK complexes preventing the transition from G1 phase to S phase in the cell division cycle.
Biological function summary	The function of p27 KIP1 involves its role in controlling cell growth and division. It achieves this by becoming a part of larger protein complexes involving CDKs and cyclins. By directly interacting with these complexes p27 KIP1 modulates cell cycle progression therefore acting as a brake on cellular proliferation. The protein is important in maintaining proper cell cycle checkpoints and preventing uncontrolled cell growth which is essential for normal cellular functioning.
Pathways	The involvement of p27 KIP1 centers on cell cycle regulation and signaling pathways such as the PI3K/AKT pathway. Its interaction with CDKs and cyclins situates it within the core mechanisms that determine cell division timing. p27 KIP1 operates alongside other proteins like cyclin D and CDK4/6 fitting into the regulatory intricacies of these pathways. Proper functioning of these pathways ensures cellular homeostasis and prevents the development of oncogenic processes.
Associated diseases and disorders	Dysfunction of p27 KIP1 has links to cancer and neurodegenerative diseases. Low levels or mutations can lead to uncontrolled cell proliferation contributing to the development and progression of cancers such as breast cancer. p27 KIP1's role in neurodegenerative diseases involves its regulation of neuronal cell cycle re-entry with abnormalities potentially exacerbating conditions like Alzheimer's disease. In these contexts its interaction with proteins such as cyclin E and CDK2 becomes particularly relevant in understanding disease mechanisms.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

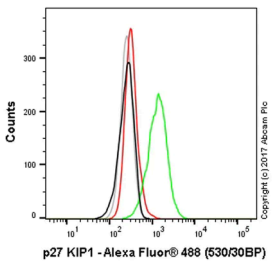
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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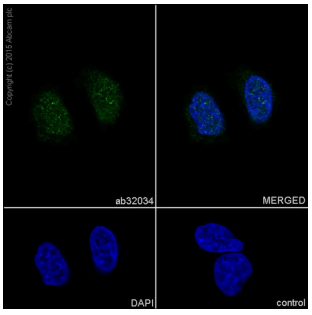
16 product images



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

P27 KIP 1 Flow Cytometry (Intracellular) staining using rabbit Anti-p27 KIP 1 antibody

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).

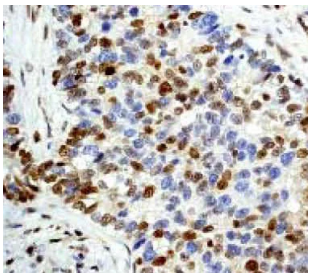


Immunocytochemistry/ Immunofluorescence - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

P27 KIP 1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p27 KIP 1 antibody

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.

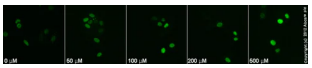
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32034).



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

P27 KIP 1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-p27 KIP 1 antibody

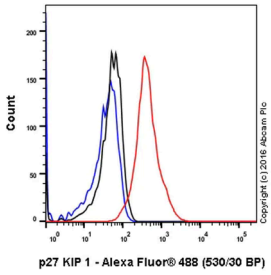
Immunohistochemical analysis of paraffin-embedded human breast carcinomaThis data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32034).



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

P27 KIP 1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p27 KIP 1 antibody

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).



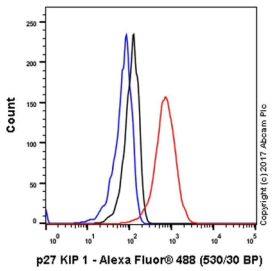
Flow Cytometry (Intracellular) - Anti-p27 KIP 1 antibody [Y236] - BSA and Azide free (ab206927)

P27 KIP 1 Flow Cytometry (Intracellular) staining using rabbit Anti-p27 KIP 1 antibody

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)

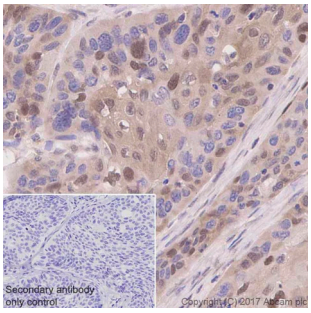
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32034).



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

P27 KIP 1 Flow Cytometry (Intracellular) staining using rabbit Anti-p27 KIP 1 antibody

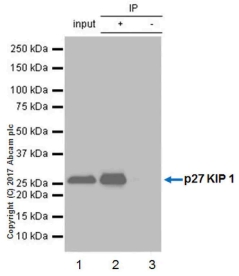
Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling p27 KIP 1 with purified ab32034 at 1/50 dilution (10 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary 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).



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

P27 KIP 1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-p27 KIP 1 antibody

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.



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

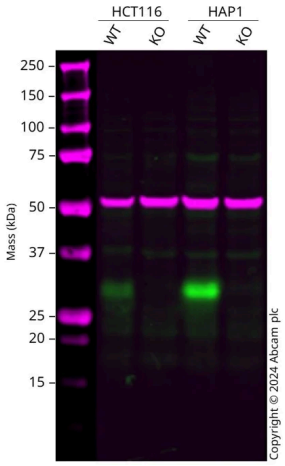
ab32034 (purified) at 1:20 dilution (2µg) immunoprecipitating p27 KIP 1 in MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate.

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).

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

Predicted band size: 22 kDa



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

P27 KIP 1 Western blot staining using rabbit Anti-p27 KIP 1 antibody

Western blot: Anti-CDKN1B antibody [Y236] (ab32034) staining at 1/5000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab32034 was shown to bind specifically to CDKN1B. A band was observed at 30 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in CDKN1B knockout cell line. To generate this image, wild-type and CDKN1B knockout HCT 116 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-p27 KIP 1 antibody [Y236] (ab32034) at 1/5000 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
CDKN1B knockout HCT 116 cell lysate at 20 µg

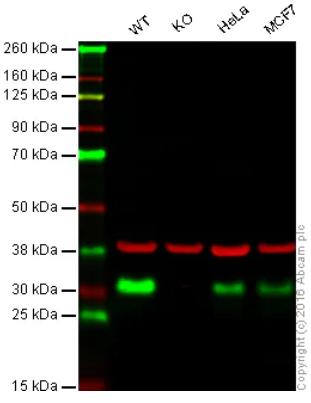
Lane 3:
Wild-type HAP1 cell lysate at 20 µg

Lane 4:
CDKN1B knockout HAP1 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.



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

P27 KIP 1 Western blot staining using rabbit Anti-p27 KIP 1 antibody

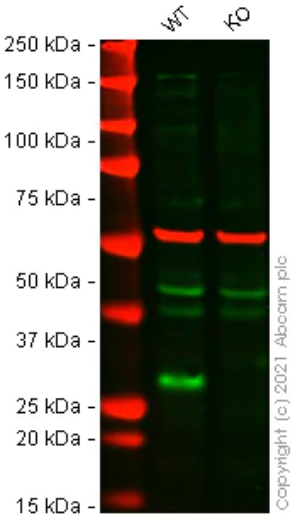
This WB data was generated using the same anti-p27 KIP 1 antibody clone, Y236, in a different buffer formulation (cat# ab32034).
Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: CDKN1B knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: MCF7 whole cell lysate (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.

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

Predicted band size: 22 kDa



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

P27 KIP 1 Western blot staining using rabbit Anti-p27 KIP 1 antibody

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

Lane 1:
Wild-type RAW 264.7 cell lysate at 20 µg

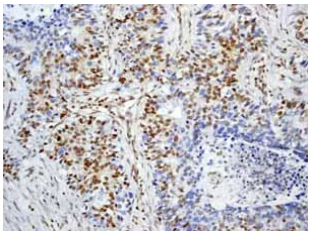
Lane 2:
CDKN1B knockout RAW 264.7 cell lysate at 20 µg

Lane 2:
Western blot - Mouse CDKN1B (p27 KIP 1) knockout RAW 264.7 cell line (ab281619)

Performed under reducing conditions.

Predicted band size: 22 kDa

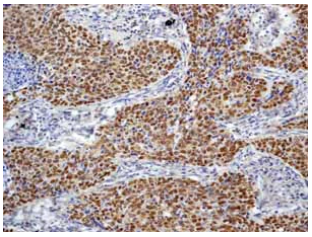
Observed band size: 28 kDa



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.

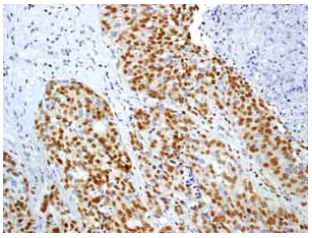
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32034).



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.

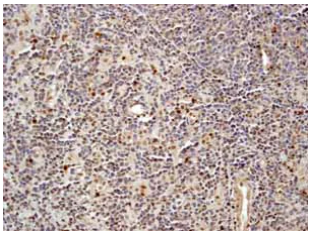
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32034).



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

Unpurified ab32034 showing positive staining in Ovarian carcinoma tissue.

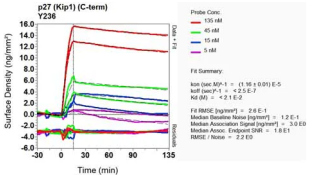
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32034).



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

Unpurified ab32034 showing positive staining in Glioma 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)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.