abcam

ab247904

Anti-KAP1 antibody [EPR5249] - 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	EPR5249
Purification technique	Affinity purification Protein A
Specificity	Although the immunogen is from a phosphor-peptide, this antibody detects phospho and non-phospho KAP1. Based on a peptide blocking experiment it has been found that the signal generated after non-phospho peptide blocking became much weaker, thus indicating that this antibody shows cross-reactivity with the non-phospho KAP1 at high level.
Concentration	1.023 - 1.024 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	For antigen retrieval, heat up to 98 degree C, below boiling, and then let cool for 10-20 minutes.

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Target data

See full target information TRIM28

Function	Nuclear corepressor for KRAB domain-containing zinc finger proteins (KRAB-ZFPs). Mediates gene silencing by recruiting CHD3, a subunit of the nucleosome remodeling and deacetylation (NuRD) complex, and SETDB1 (which specifically methylates histone H3 at 'Lys-9' (H3K9me)) to the promoter regions of KRAB target genes. Enhances transcriptional repression by coordinating the increase in H3K9me, the decrease in histone H3 'Lys-9 and 'Lys-14' acetylation (H3K9ac and H3K14ac, respectively) and the disposition of HP1 proteins to silence gene expression. Recruitment of SETDB1 induces heterochromatinization. May play a role as a coactivator for CEBPB and NR3C1 in the transcriptional activation of ORM1. Also a corepressor for ERBB4. Inhibits E2F1 activity by stimulating E2F1-HDAC1 complex formation and inhibiting E2F1 acetylation. May serve as a partial backup to prevent E2F1-mediated apoptosis in the absence of RB1. Important regulator of CDKN1A/p21(CIP1). Has E3 SUMO-protein ligase activity toward itself via its PHD-type zinc finger. Also specifically sumoylates IRF7, thereby inhibiting its transactivation activity. Ubiquitinates p53/TP53 leading to its proteasomal degradation; the function is enhanced by MAGEC2 and MAGEA2, and possibly MAGEA3 and MAGEA6. Mediates the nuclear localization of KOX1, ZNF268 and ZNF300 transcription factors. In association with isoform 2 of ZFP90, is required for the transcriptional repressor activity of FOXP3 and the suppressive function of regulatory T-cells (Treg) (PubMed:23543754). Probably forms a corepressor complex required for activated KRAS-mediated promoter hypermethylation and transcriptional silencing of tumor suppressor genes (TSGs) or other tumor-related genes in colorectal cancer (CRC) cells (PubMed:24623306). Required to maintain a transcriptionally repressive state of genes in undifferentiated embryonic stem cells (ESCs) (PubMed:24623306). In ESCs, in collaboration with SETDB1, is also required for H3K9me3 and silencing of endogenous and introduced retroviruses in a DNA-methylation independent-pathway (By similarity). Associates at promoter regions of tumor suppressor genes (TSGs) leading to their gene silencing (PubMed:24623306). The SETDB1-TRIM28-ZNF274 complex may play a role in recruiting ATRX to the 3'-exons of zinc-finger coding genes with atypical chromatin signatures to establish or maintain/protect H3K9me3 at these transcriptionally active regions (PubMed:27029610). (Microbial infection) Plays a critical role in the shutdown of lytic gene expression during the early stage of herpes virus 8 primary infection. This inhibition is mediated through interaction with herpes virus 8 protein LANA1.

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

ab247904 is the carrier-free version of ab109545.

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	KAP1 also known as TRIM28 or TIF1β is a multifunctional protein involved in transcriptional regulation. It has a molecular weight of approximately 87 kDa. This protein is expressed in a wide variety of human tissues where it plays a role as a co-repressor for KRAB domain-containing zinc finger proteins. KAP1 recruits various epigenetic modifiers to silence transcription and this activity depends on its phosphorylation status. You might explore KAP1 using KAP1 ELISA kits and study its post-translational modifications like phosphorylation in various conditions.
Biological function summary	KAP1 acts as a scaffold protein facilitating the assembly of large protein complexes that include chromatin remodelers and histone deacetylases. It is significant in maintaining chromatin structure and mediating gene silencing. KAP1 phosphorylation serves as a switch between its roles in transcriptional repression and activation. This protein also interacts with heterochromatin protein 1 (HP1) and other TRIM family proteins playing an important role in genomic stability by controlling gene expression and repair mechanisms.

Pathways	KAP1 participates actively in p53 and DNA damage response pathways. In the p53 pathway KAP1 regulates genes involved in the cell cycle and apoptosis acting in conjunction with the p53 protein. Within the DNA damage response KAP1 modulates the repair of double-strand breaks by interacting with proteins like ATM kinase influencing cellular sensitivity to genotoxic stress. These pathways highlight its importance in maintaining cellular homeostasis and response to external stimuli.
Associated diseases and disorders	KAP1 has known connections with cancer and neurological disorders due to its regulatory effects on gene expression and genome stability. In cancer aberrant KAP1 activity can lead to unregulated cell proliferation and survival often involving changes in its interaction with the p53 protein. In neurological disorders dysregulation of KAP1 is associated with impaired neural development and neurodegeneration. Altogether these associations highlight KAP1 as a potential target for therapeutic interventions in related diseases.

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

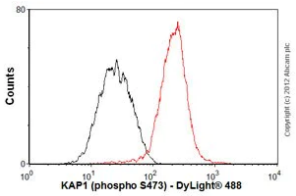
We do not recommend this combination. It is not covered by our product promise.

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.

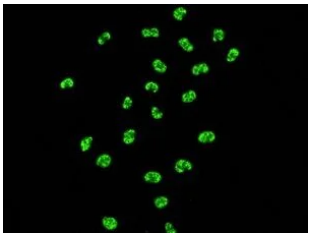
8 product images



Flow Cytometry (Intracellular) - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 Flow Cytometry (Intracellular) staining using rabbit Anti-KAP1 antibody

This data was developed using ab109545, the same antibody clone in a different buffer formulation:
Overlay histogram showing HeLa cells stained with ab109545 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab190545, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunocytochemistry/ Immunofluorescence - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-KAP1 antibody

This data was developed using ab109545, the same antibody clone in a different buffer formulation:
ab109545, at a 1/100 dilution, staining KAP1 in HeLa cells

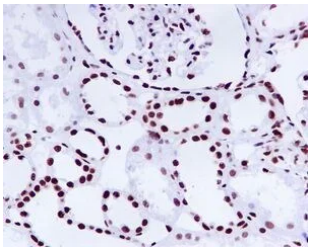


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-KAP1 antibody

This data was developed using ab109545, the same antibody clone in a different buffer formulation:
ab109545, at a 1/100 dilution, staining KAP1 in formalin-fixed, paraffin-embedded Human colon tissue.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

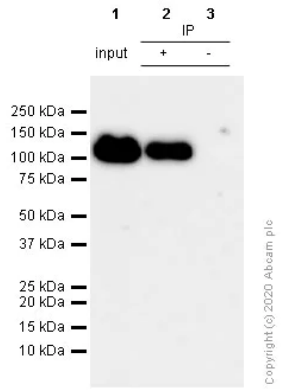


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-KAP1 antibody

This data was developed using ab109545, the same antibody clone in a different buffer formulation:
ab109545, at a 1/100 dilution, staining KAP1 in formalin-fixed, paraffin-embedded Human kidney tissue.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.



Immunoprecipitation - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 was immunoprecipitated from 0.35 mg A431 (Human epidermoid carcinoma epithelial cell) cell lysate 10 µg with ab109545 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab109545 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.
Lane 1: A431 (Human epidermoid carcinoma epithelial cell) cell lysate 10 µg

Lane 2: ab109545 IP in A431 cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab109545 in A431 cell lysate

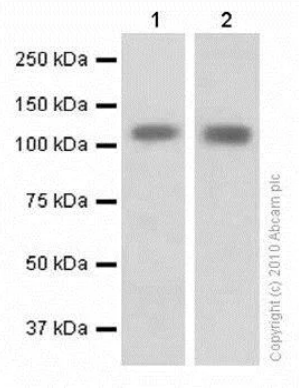
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 32 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109545).

All lanes:
Immunoprecipitation - Anti-KAP1 antibody [EPR5249] (ab109545)

Predicted band size: 88 kDa



Western blot - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 Western blot staining using rabbit Anti-KAP1 antibody

This data was developed using ab109545, the same antibody clone in a different buffer formulation:
Blocking/diluting buffer and concentration: 5% NFDM/TBST

All lanes:
Western blot - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904) at 1/10000 dilution

Lane 1:
A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates at 15 µg

Lane 2:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates at 15 µg

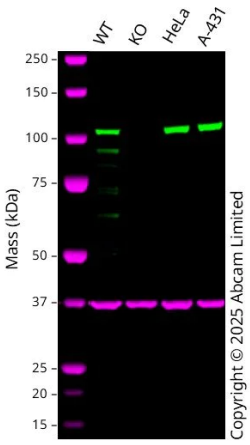
Secondary

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

Developed using the ECL technique.

Predicted band size: 88 kDa

Exposure time: 10s



Western blot - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 Western blot staining using rabbit Anti-KAP1 antibody

This data was developed using ab109545, the same antibody clone in a different buffer formulation.

Western blot: Anti-KAP1 antibody [EPR5249] ab109545 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 110 kDa in Wild-type HCT 116 cell lysates with no signal observed at this size in TRIM28 knockout HCT 116 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc 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 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-KAP1 antibody [EPR5249] (ab109545) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 at 20 µg

Lane 2:
Western blot - Human TRIM28 knockout HCT116 cell line (ab289073) at 20 µg

Lane 3:
HeLa at 20 µg

Lane 4:
A-431 at 20 µg

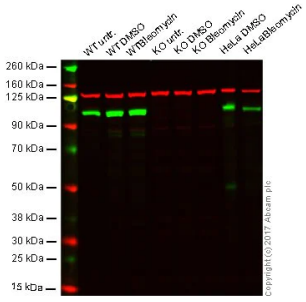
Secondary

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

Performed under reducing conditions.

Predicted band size: 89 kDa

Observed band size: 110 kDa, 37 kDa



Western blot - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

KAP1 Western blot staining using rabbit Anti-KAP1 antibody

This data was developed using ab109545, the same antibody clone in a different buffer formulation:
Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: HAP1 + DMSO whole cell lysate (20 µg)
Lane 3: HAP1 + Blaomycin whole cell lysate (20 µg)
Lane 4: TRIM28 knockout HAP1 whole cell lysate (20 µg)
Lane 5: TRIM28 knockout HAP1 + DMSO whole cell lysate (20 µg)
Lane 6: TRIM28 knockout HAP1 + Blaomycin whole cell lysate (20 µg)
Lane 7: HeLa + DMSO whole cell lysate (20 µg)
Lane 8: HeLa + Blaomycin whole cell lysate (20 µg)

Lanes 1 - 8: Merged signal (red and green). Green - ab109545 observed at 110 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab109545 was shown to specifically react with KAP1 in wild type cells as signal was lost in KAP1 knockout cells. Wild-type and KAP1 knockout samples were subjected to SDS-PAGE. ab109545 and ab8245 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 10000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.
All lanes:
Western blot - Anti-KAP1 antibody [EPR5249] - BSA and Azide free (ab247904)

Predicted band size: 88 kDa

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