

## Product datasheet

### Anti-KAP1 antibody [EPR5249] ab109545

KO **VALIDATED** Recombinant RabMAb<sup>®</sup>

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#### Overview

<b>Product name</b>	Anti-KAP1 antibody [EPR5249]
<b>Description</b>	Rabbit monoclonal [EPR5249] to KAP1
<b>Host species</b>	Rabbit
<b>Specificity</b>	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 ab109545 shows cross-reactivity with the non-phospho KAP1 at high level.
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), IP, WB, IHC-P, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: A431 and MCF7 cell lysates; IHC-P: Human colon and kidney tissue; ICC/IF: Hela cells; IP: A431 cell lysate.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	<p>pH: 7.20</p> <p>Preservative: 0.05% Sodium azide</p> <p>Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant</p>

<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR5249
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab109545 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
<b>Flow Cyt (Intra)</b>		1/100. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
<b>IP</b>		1/30.
<b>WB</b>	★ ★ ★ ★ ★ (1)	1/10000 - 1/50000. Detects a band of approximately 110 kDa (predicted molecular weight: 89 kDa).
<b>IHC-P</b>		1/100 - 1/250. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. Heat up to 98 degrees C, below boiling, and then let cool for 10-20 min.
<b>ICC/IF</b>		1/100 - 1/250.

## Target

<b>Function</b>	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 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.
<b>Tissue specificity</b>	Expressed in all tissues tested including spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes.
<b>Pathway</b>	Protein modification; protein sumoylation.
<b>Sequence similarities</b>	Belongs to the TRIM/RBCC family. Contains 2 B box-type zinc fingers. Contains 1 bromo domain.

Contains 1 PHD-type zinc finger.  
Contains 1 RING-type zinc finger.

## Domain

The HP1 box is both necessary and sufficient for HP1 binding.

The PHD-type zinc finger enhances CEBPB transcriptional activity. The PHD-type zinc finger, the HP1 box and the bromo domain, function together to assemble the machinery required for repression of KRAB domain-containing proteins. Acts as an intramolecular SUMO E3 ligase for autosumoylation of bromodomain.

The RING-finger-B Box-coiled-coil/tripartite motif (RBCC/TRIM motif) is required for interaction with the KRAB domain of KRAB-zinc finger proteins. Binds four zinc ions per molecule. The RING finger and the N-terminal of the leucine zipper alpha helical coiled-coil region of RBCC are required for oligomerization.

Contains one Pro-Xaa-Val-Xaa-Leu (PxVxL) motif, which is required for interaction with chromoshadow domains. This motif requires additional residues -7, -6, +4 and +5 of the central Val which contact the chromoshadow domain.

## Post-translational modifications

Phosphorylated upon DNA damage, probably by ATM or ATR. ATM-induced phosphorylation on Ser-824 represses sumoylation leading to the de-repression of expression of a subset of genes involved in cell cycle control and apoptosis in response to genotoxic stress. Dephosphorylation by the phosphatases, PPP1CA and PP1CB forms, allows sumoylation and expression of TRIM28 target genes.

Sumoylation/desumoylation events regulate TRIM28-mediated transcriptional repression.

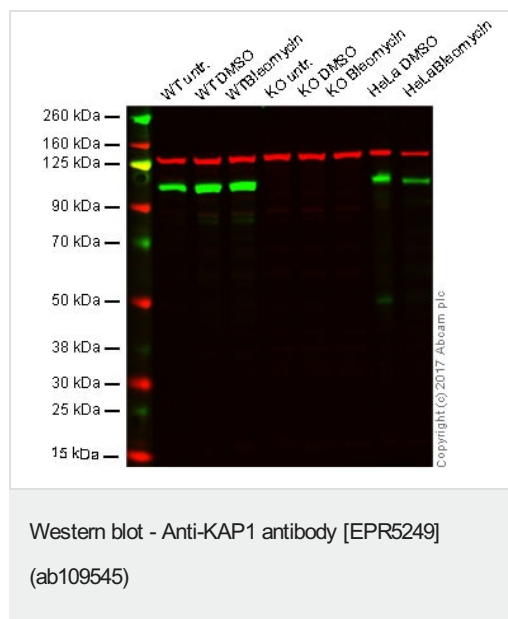
Sumoylation is required for interaction with CHD3 and SETDB1 and the corepressor activity.

Represses and is repressed by Ser-824 phosphorylation. Enhances the TRIM28 corepressor activity, inhibiting transcriptional activity of a number of genes including GADD45A and CDKN1A/p21. Lys-554, Lys-779 and Lys-804 are the major sites of sumoylation. In response to Dox-induced DNA damage, enhanced phosphorylation on Ser-824 prevents sumoylation and allows de-repression of CDKN1A/p21.

## Cellular localization

Nucleus. Associated with centromeric heterochromatin during cell differentiation through CBX1.

## Images



**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)

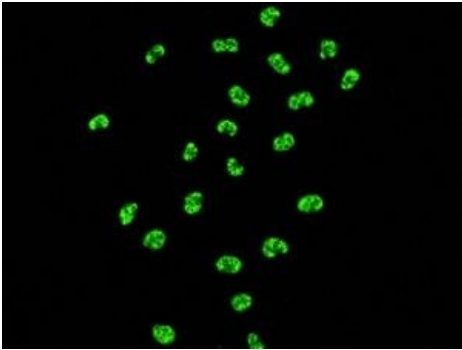
**Lane 7:** HeLa + DMSO whole cell lysate (20 µg)

**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.

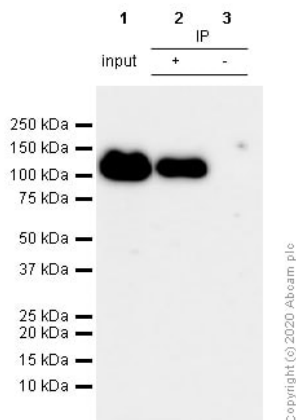
ab109545 was shown to specifically react with KAP1 in wild type cells as signal was lost in KAP1 knockout cells. Wild-type and KAP1 knockout samples were subjected to SDS-PAGE. ab109545 and [ab8245](#) (Mouse anti-GAPDH loading control) were incubated

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.



Immunocytochemistry/ Immunofluorescence - Anti-KAP1 antibody [EPR5249] (ab109545)

ab109545, at a 1/100 dilution, staining KAP1 in HeLa cells



Immunoprecipitation - Anti-KAP1 antibody [EPR5249] (ab109545)

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.

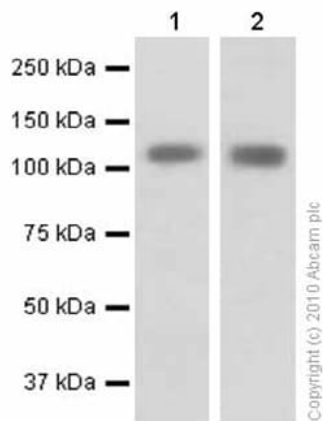
Lane 1: A431 (Human epidermoid carcinoma epithelial cell) cell lysate 10 µg

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



Western blot - Anti-KAP1 antibody [EPR5249] (ab109545)

**All lanes :** Anti-KAP1 antibody [EPR5249] (ab109545) at 1/10000 dilution

**Lane 1 :** A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates

**Lane 2 :** MCF7 (Human breast adenocarcinoma epithelial 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 (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Developed using the ECL technique.

**Predicted band size:** 89 kDa

**Observed band size:** 110 kDa

**Exposure time:** 10 seconds

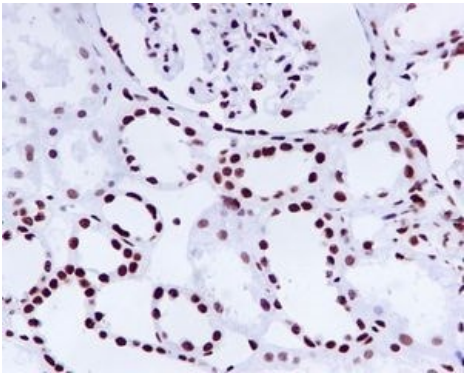
Blocking/diluting buffer and concentration: 5% NFDM/TBST

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.



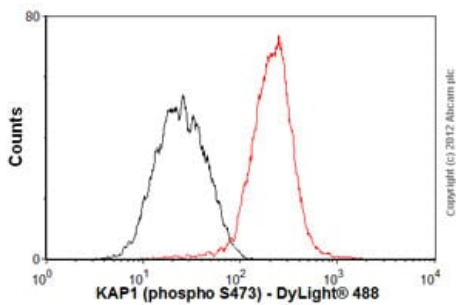
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 antibody [EPR5249] (ab109545)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 antibody [EPR5249] (ab109545)

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.



Flow Cytometry (Intracellular) - Anti-KAP1 antibody [EPR5249] (ab109545)

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 (**ab109545**, 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) (1 µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed.

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-KAP1 antibody [EPR5249] (ab109545)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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