

Product datasheet

Anti-KAP1 (phospho S824) antibody [EPR5248] ab133440

KO VALIDATED Recombinant RabMAb

★★★★★ [1 Abreviews](#) [9 References](#) [6 Images](#)

Overview

Product name	Anti-KAP1 (phospho S824) antibody [EPR5248]
Description	Rabbit monoclonal [EPR5248] to KAP1 (phospho S824)
Host species	Rabbit
Tested applications	Suitable for: WB, IP, Dot blot
Species reactivity	Reacts with: Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: WT HAP1 cell lysate (+/- Bleomycin); HeLa cell lysate (+/- Bleomycin). Dot blot: KAP1 (phospho S824) phospho peptide. IP: HeLa treated with 3uM etoposide for 1h whole cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS</p>
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5248
Isotype	IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab133440 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
WB		1/1000. Detects a band of approximately 110 kDa (predicted molecular weight: 88 kDa). For unpurified use at 1:50000-1:200000 dilution range
IP		1/10 - 1/100.
Dot blot		Use at an assay dependent concentration.

Target

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 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.

Tissue specificity

Expressed in all tissues tested including spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes.

Pathway

Protein modification; protein sumoylation.

Sequence similarities

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**

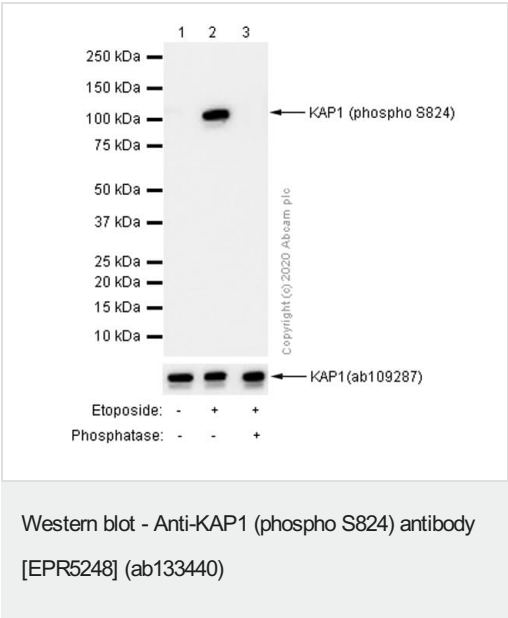
Phosphorylated upon DNA damage, probably by ATM or ATR. ATM-induced phosphorylation on Ser-824 represses sumoylation leading to the de-repression of expression of a subset of genes involved in cell cycle control and apoptosis in response to genotoxic stress. Dephosphorylation by the phosphatases, PPP1CA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



All lanes : Anti-KAP1 (phospho S824) antibody [EPR5248] (ab133440) at 1/1000 dilution (Purified)

Lane 1 : Untreated NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

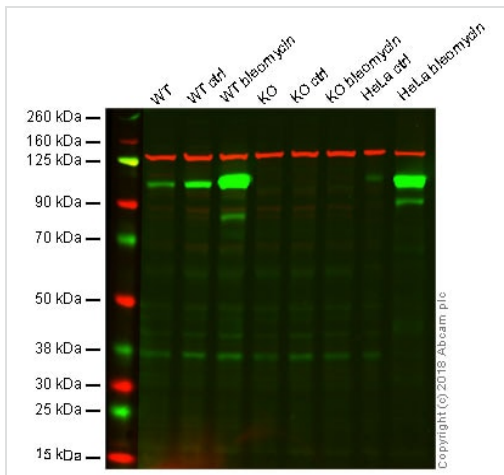
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) treated with 3 μ M etoposide for 1 hour whole cell lysate

Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) treated with 3 μ M etoposide for 1 hour whole cell lysate, then the membrane treated with Alkaline Phosphatase for 1 hour

Secondary

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

Predicted band size: 88 kDa



Western blot - Anti-KAP1 (phospho S824) antibody [EPR5248] (ab133440)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

Lane 2: Wild type HAP1 + DMSO whole cell lysate (20 µg)

Lane 3: Wild type HAP1 + Bleomycin whole cell lysate (20 µg)

Lane 4: KAP1 knockout HAP1 whole cell lysate (20 µg)

Lane 5: KAP1 knockout HAP1 + DMSO whole cell lysate (20 µg)

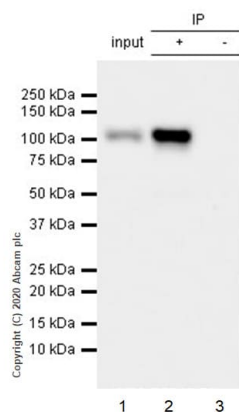
Lane 6: KAP1 knockout HAP1 + Bleomycin whole cell lysate (20 µg)

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

Lane 8: HeLa + Bleomycin whole cell lysate (20 µg)

Lanes 1 - 8: Merged signal (red and green). Green - ab133440 observed at 105 kDa. Red - loading control, **ab130007**, observed at 125 kDa.

ab133440 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. ab133440 and **ab130007** (Mouse anti-vinculin loading control) were incubated overnight at 4°C both at a 1/20000 dilution. 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. Treated with 30 µg/mL Bleomycin in DMSO for 30 minutes.



Immunoprecipitation - Anti-KAP1 (phospho S824) antibody [EPR5248] (ab133440)

Purified ab133440 at 1/50 dilution (2µg) immunoprecipitating

KAP1 in HeLa treated with 3uM etoposide for 1h whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) treated with 3uM etoposide for 1h whole cell lysate 10µg

Lane 2 (+): ab133440 + HeLa treated with 3uM etoposide for 1h whole cell lysate.

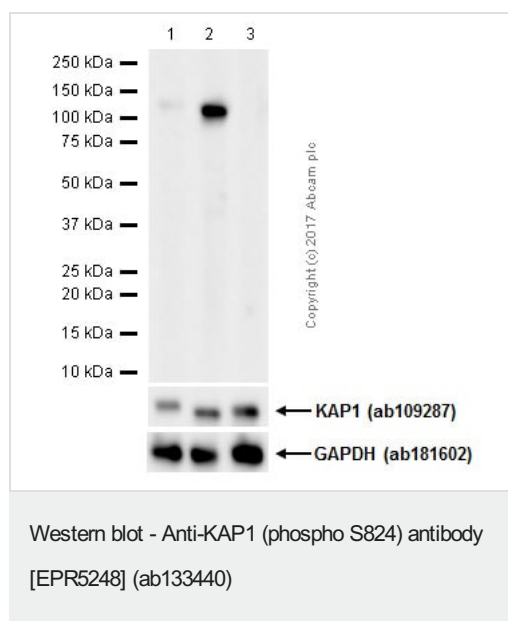
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32132** in HeLa treated with 3uM etoposide for 1h whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 110 kDa



All lanes : Anti-KAP1 (phospho S824) antibody [EPR5248] (ab133440) at 1/1000 dilution (Unpurified)

Lane 1 : MDA-MB-231 (human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : MDA-MB-231 treated with 1 μM camptothecin for 24h whole cell lysate

Lane 3 : MDA-MB-231 treated with 1 μM camptothecin for 24h whole cell lysate. Then the membrane was incubated with alkaline phosphatase

Lysates/proteins at 10 μg per lane.

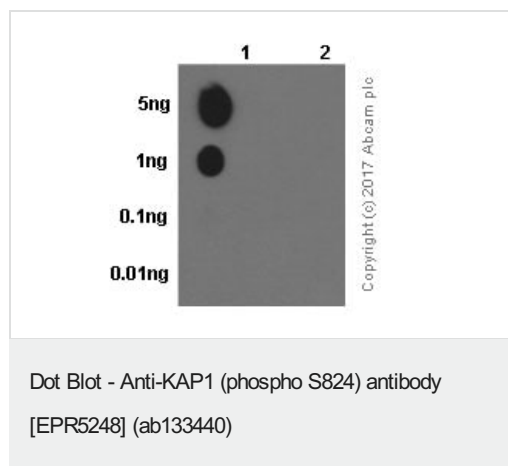
Secondary

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

Predicted band size: 88 kDa

Observed band size: 110 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Dot blot analysis of KAP1 (phospho S824) phospho peptide (Lane 1) and KAP1 non-phospho peptide (Lane 2) labelling KAP1 (phospho S824) with Unpurified ab133440 at a dilution of 1/1000. A Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) was used as the secondary antibody at a dilution of 1/20,000. Blocking buffer: 5% NFDM/TBST. Dilution buffer: 5% NFDM /TBST.

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 (phospho S824) antibody [EPR5248]
(ab133440)

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