abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 2 References 5 Images

Overview

Product name	Anti-KAP1 (phospho S824) antibody [EPR5248]
Description	Rabbit monoclonal [EPR5248] to KAP1 (phospho S824)
Host species	Rabbit
Specificity	ab133440 only detects KAP1 phosphorylated at Serine 824.
Tested applications	**Suitable for:** WB, IP, Dot blot **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic phospho peptide corresponding to a region surrounding Serine 824 of Human KAP1 (UniProt Q13263).
Positive control	Lysate from HeLa cells treated with gamma radiation.
General notes	Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.40 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol, 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	EPR5248
Isotype	IgG

Applications

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		1/50000 - 1/200000. Detects a band of approximately 110 kDa (predicted molecular weight: 88 kDa).
IP		1/10 - 1/100.
Dot blot		Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF.

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.

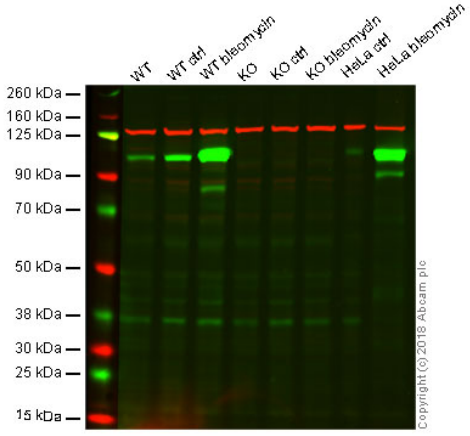
Post-translational modifications

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

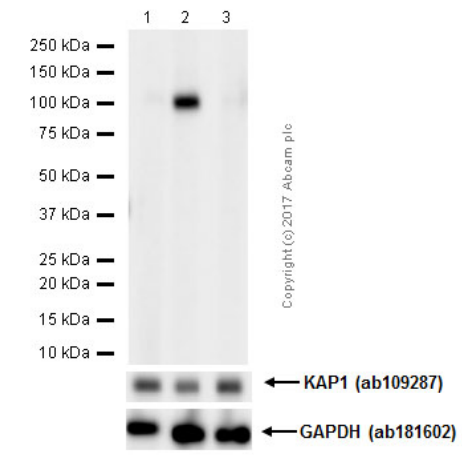


Western blot - Anti-KAP1 (phospho S824) antibody [EPR5248] (ab133440)

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.



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

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

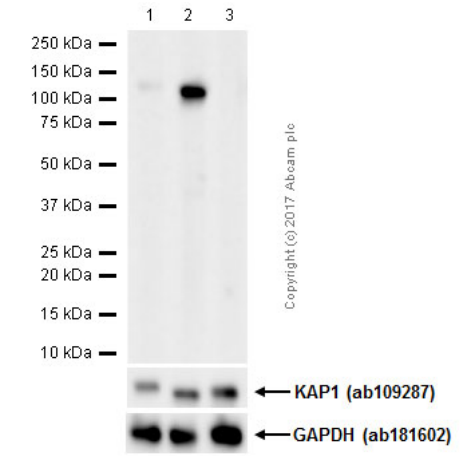
Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa treated with 3µM etoposide for 1h whole cell lysate
Lane 3 : HeLa treated with 3µM etoposide for 1h whole cell lysate. Then the membrane was incubated with alkaline phosphatase.

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
why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST.



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

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

Lane 1 : MDA-MB-231 (human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : MDA-MB-231 treated with 1µM camptothecin for 24h whole cell lysate
Lane 3 : MDA-MB-231 treated with 1µM 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

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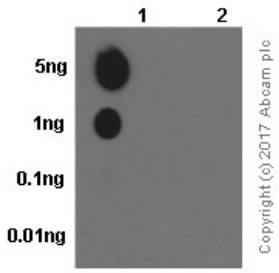
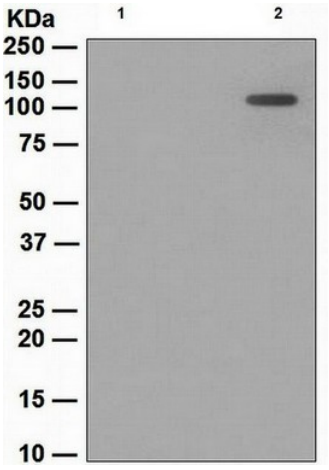


Dot Blot - Anti-KAP1 (phospho S824) antibody [EPR5248] (ab133440)

Dot blot analysis of KAP1 (phospho S824) phospho peptide (Lane 1) and KAP1 non-phospho peptide (Lane 2) labelling KAP1 (phospho S824) with 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.



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

All lanes : Anti-KAP1 (phospho S824) antibody [EPR5248] (ab133440) at 1/50000 dilution

Lane 1 : Lysate of HeLa cells, untreated
Lane 2 : Lysate of HeLa cells treated with gamma radiation

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP conjugated goat anti-rabbit at 1/2000 dilution

Predicted band size: 88 kDa

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