abcam

Product datasheet

Anti-KAP1 (phospho S824) antibody [EPR5248] - BSA and Azide free ab215549

KO VALIDATED Recombinant RabMAb

5 Images

Overview

Product name	Anti-KAP1 (phospho S824) antibody [EPR5248] - BSA and Azide free
Description	Rabbit monoclonal [EPR5248] to KAP1 (phospho S824) - BSA and Azide free
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	ab215549 is the carrier-free version of ab133440. Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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 production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5248
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab215549 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

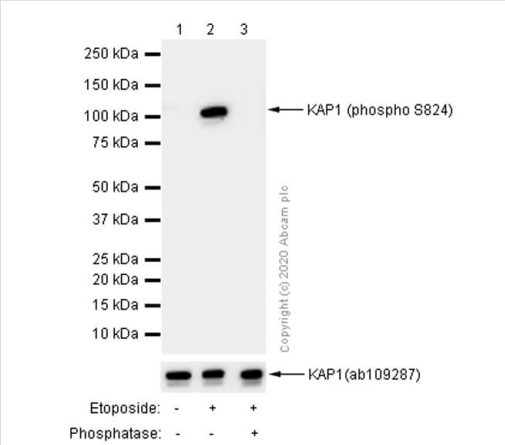
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 110 kDa (predicted molecular weight: 88 kDa).
IP		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.

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



Western blot - Anti-KAP1 (phospho S824) antibody [EPR5248] - BSA and Azide free (ab215549)

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

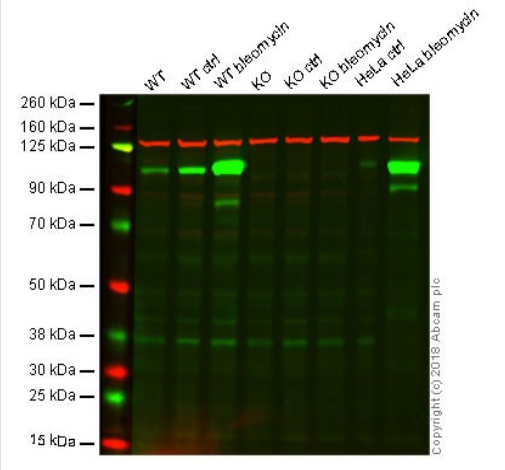
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) treated with 3ÂµM etoposide for 1 hour whole cell lysate

Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) treated with 3ÂµM 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] - BSA and Azide free (ab215549)

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.

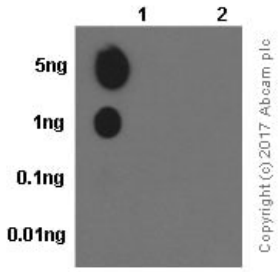
ab133440 was shown to specifically react with KAP1 in wild type cells as signal was lost in KAP1 knockout cells. Wild-type and KAP1 knockout samples were subjected to SDS-PAGE. ab133440 and ab130007 (Mouse anti-vinculin loading control) were incubated overnight at 4°C both at a 1/20000 dilution. Blots 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133440).

Immunoprecipitation - Anti-KAP1 (phospho S824) antibody [EPR5248] - BSA and Azide free (ab215549)

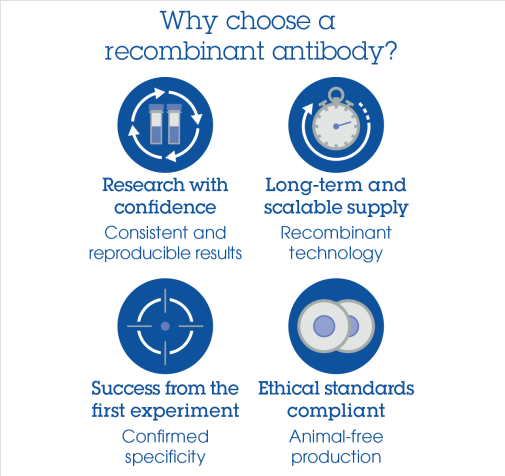
This data was developed using ab133440, the same antibody clone in a different buffer formulation.
Purified ab133440 at 1/50 dilution (2µg) immunoprecipitating KAP1 in HeLa treated with 3uM etoposide for 1h whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) treated 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



Dot Blot - Anti-KAP1 (phospho S824) antibody [EPR5248] - BSA and Azide free (ab215549)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133440)



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