abcam

Product datasheet

Anti-KAP1 antibody ab10483

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Overview

Product name Anti-KAP1 antibody

Description Rabbit polyclonal to KAP1

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. The immunogen is between aa 1-50.

Database link: Q13263

Positive control WB: human HeLa, HEK293T cells; mouse NIH3T3, TCMK-1, 4T1, CT26.WT, rat C6 cells. IP:

HeLa cells. IHC: human breast carcinoma

General notesThe Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7

Preservative: 0.1% Sodium azide

Constituents: 0.021% PBS, 1.764% Sodium citrate, 1.815% Tris

Purity Immunogen affinity purified

Purification notesAntibodies were affinity purified using the peptide immobilized on solid support.

Clonality Polyclonal

Isotype IgG

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The Abpromise guarantee

Our Abpromise guarantee covers the use of ab10483 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	★★★★☆(3)	1/2000 - 1/10000. Detects a band of approximately 110 kDa (predicted molecular weight: 100 kDa).
IHC-P	**** <u>(1)</u>	1/500 - 1/2000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP	★★★★★ (1)	Use at 2-10 μg/mg of lysate.

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.

Expressed in all tissues tested including spleen, thymus, prostate, testis, ovary, small intestine,

Tissue specificity

Protein modification; protein sumoylation.

Sequence similarities

Belongs to the TRIM/RBCC family.
Contains 2 B box-type zinc fingers.
Contains 1 brome demain.

colon and peripheral blood leukocytes.

Contains 1 bromo domain.

Contains 1 PHD-type zinc finger.

Contains 1 RING-type zinc finger.

Domain

Pathway

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

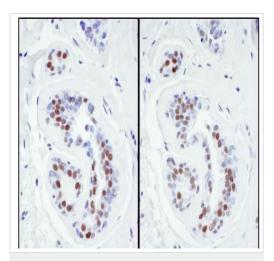
Phosphorylated upon DNA damage, probably by ATM or ATR. ATM-induced phosphorylation on Ser-824 represses sumoylation leading to the de-repression of expression of a subset of genes involved in cell cycle control and apoptosis in response to genotoxic stress. Dephosphorylation by the phosphatases, PPP1CA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

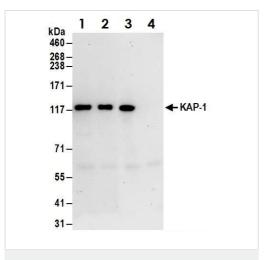
Nucleus. Associated with centromeric heterochromatin during cell differentiation through CBX1.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KAP1 antibody (ab10483)

Immunohistochemistry analysis of formalin-fixed paraffin embedded sections of human breast tissue staining KAP-1 with ab10483 at 1/1000 dilution, showing the current lot on the left, and previous lot on the right.



Immunoprecipitation - Anti-KAP1 antibody (ab10483)

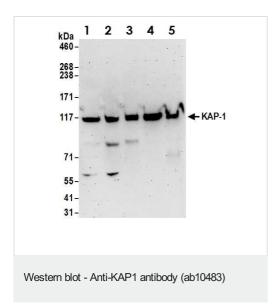
Immunoprecipitation of KAP-1 from HeLa whole cell lysate using ab10483

Lane 1: ab10483 current lot

Lane 2: ab10483 previous lot

Lane 3: other rabbit anti-KAP-1 antibody

Lane 4: Control IgG



All lanes: Anti-KAP1 antibody (ab10483) at 1 µg/ml

Lane 1 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

Lane 2 : TCMK-1 (mouse kidney epithelial cell line) whole cell lysate

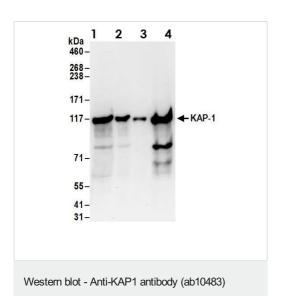
Lane 3: 4T1 (Mouse mammary gland carcinoma cell line) whole cell lysate

Lane 4: CT26.WT (murine colon carcinoma) whole cell lysate

Lane 5: C6 (rat glioma cell line) whole cell lysate

Lysates/proteins at 50 µg/ml per lane.

Predicted band size: 100 kDa



Exposure time: 3 minutes

All lanes: Anti-KAP1 antibody (ab10483) at 0.1 µg/ml

Lane 1: HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 50 µg

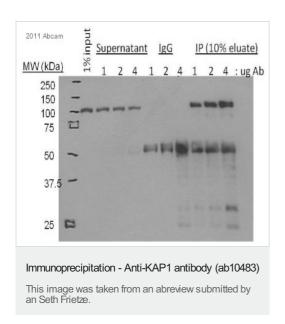
Lane 2: HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 15 µg

Lane 3 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 5 μg

Lane 4 : HEK293T cells (human epithelial cell line from embryonic kidney transformed with large T antigen) at $50 \mu g$

Predicted band size: 100 kDa

Exposure time: 30 seconds



The ab10483 antibody was used to immunoprecipitate KAP1 from HEK293 nuclear extracts. Three different amounts of ab10483 or control IgG were tested (1, 2 or 4 ug). The eluates from these experiments as well as the supernatants from the KAP1 IPs were analyzed by Western blotting using the ab10483 antibody. As shown by Western blotting, the presence of a ~110 kDa band demonstrated that KAP1 was specifically precipitated from these extracts. The 50 and 25 kDa bands correspond to the IgG of the IP antibodies.

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