abcam

Product datasheet

Anti-KAP1 antibody [TRI5I093] ab50885

KO VALIDATED

4 Images

Overview

Product name	Anti-KAP1 antibody [TRI5I093]
Description	Mouse monoclonal [TRI5I093] to KAP1
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, WB, Dot blot
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment (Human)
Positive control	Purchase matching WB positive control: **Recombinant Human KAP1 protein** › NIH3T3 lysate. HeLa cells.

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 long term.
Storage buffer	Preservative: 0.05% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Protein G purified
Purification notes	Filtered through a 0.22µm membrane.
Clonality	Monoclonal
Clone number	TRI5I093
Isotype	IgG1

Applications

Our Abpromise guarantee covers the use of **ab50885** 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
ICC/IF		1/5.
WB		1/50. Predicted molecular weight: 89 kDa.
Dot blot		Use at an assay dependent dilution.

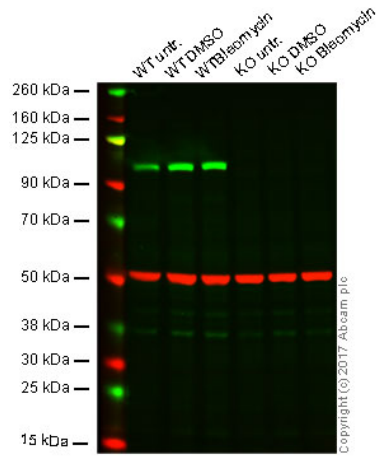
Target

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

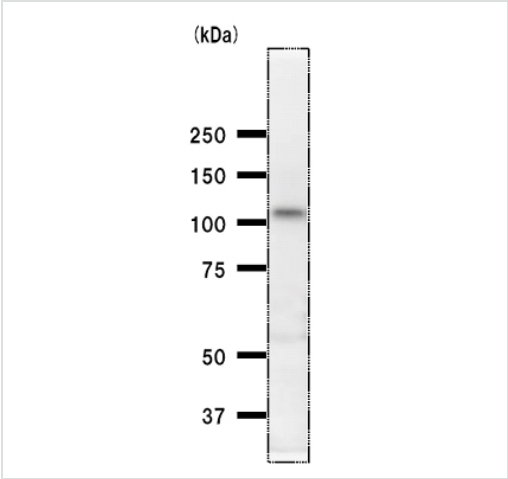


Western blot - Anti-KAP1 antibody [TRI5I093] (ab50885)

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 + Blaomycin 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 + Blaomycin whole cell lysate (20 µg)

Lanes 1 - 6: Merged signal (red and green). Green - ab50885 observed at 110 kDa. Red - loading control, ab181602, observed at 37 kDa.

ab50885 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. ab50885 and ab181602 (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 50 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.

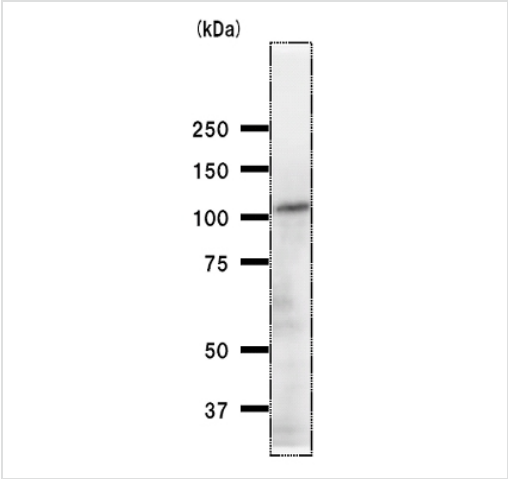


Western blot - KAP1 antibody [TRI5I093] (ab50885)

Anti-KAP1 antibody [TRI5I093] (ab50885) at 1/50 dilution + HT1080 whole cell lysate at 25 µg

Secondary
Anti mouse IgG antibody at 1/2500 dilution

Predicted band size: 89 kDa
Observed band size: 110 kDa

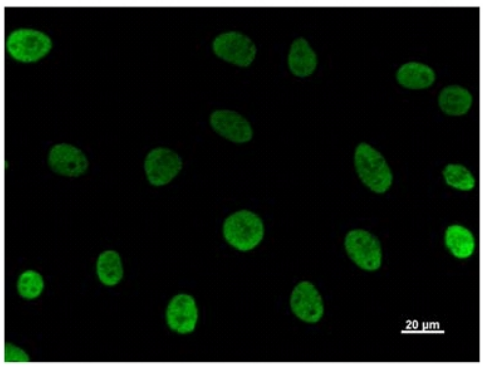


Western blot - KAP1 antibody [TRI5I093] (ab50885)

Anti-KAP1 antibody [TRI5I093] (ab50885) at 1/50 dilution + NIH3T3 whole cell lysate at 25 µg/ml

Secondary
Anti Mouse IgG antibody at 1/2500 dilution

Predicted band size: 89 kDa
Observed band size: 110 kDa



Immunocytochemistry/ Immunofluorescence - KAP1 antibody [TRI5I093] (ab50885)

Ab50885 at 1/5 dilution staining HeLa cells; visualised with Alexa Fluor® 488 Goat Anti-mouse IgG at 1/200 dilution.

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