abcam

Product datasheet

Anti-KAP1 (phospho S824) antibody [BL-246-7B5] ab243870

Recombinant

7 Images

Overview

Product name	Anti-KAP1 (phospho S824) antibody [BL-246-7B5]
Description	Rabbit monoclonal [BL-246-7B5] to KAP1 (phospho S824)
Host species	Rabbit
Tested applications	**Suitable for:** IP, IHC-P, WB, ICC
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide corresponding to Human KAP1 (phospho S824). NP_005753.1 and Gene ID 10155. Database link: Q13263
Positive control	WB: NIH/3T3 and HEK-293T whole cell lysates. IHC-P: Human breast and lung carcinoma tissue. ICC: HeLa whole cell lysate. IP: HEK-293T cell lysate treated with 100 µM etoposide.
General notes	This product is sold under License from Bethyl Laboratories, Inc.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.8 Preservative: 0.09% Sodium azide Constituents: 98% Borate buffered saline, 0.1% BSA
Purity	Protein A purified
Purification notes	Recombinant antibody was purified from cell culture supernatant.
Clonality	Monoclonal
Clone number	BL-246-7B5
Isotype	IgG

Applications

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab243870 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
IP		Use at an assay dependent concentration. Use 20µl/mg lysate.
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		1/1000.
ICC		1/100 - 1/500. Permeabilization with Triton-X 100 is recommended for formaldehyde-fixed cells.

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.

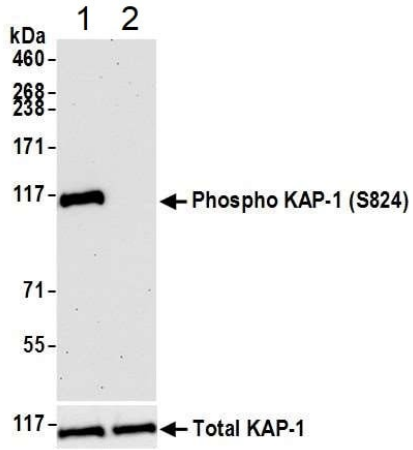
Pathway Protein modification; protein sumoylation.

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 [BL-246-7B5] (ab243870)

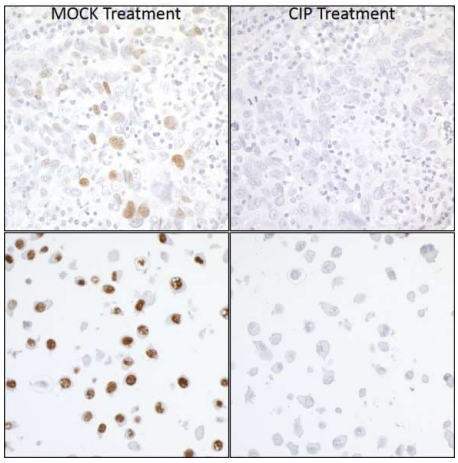
All lanes : Anti-KAP1 (phospho S824) antibody [BL-246-7B5] (ab243870) at 1/1000 dilution

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast cell line) cells treated with 100 µM etoposide (+)
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast cell line) cells, mock treated (-)

Lysates/proteins at 50 µg per lane.

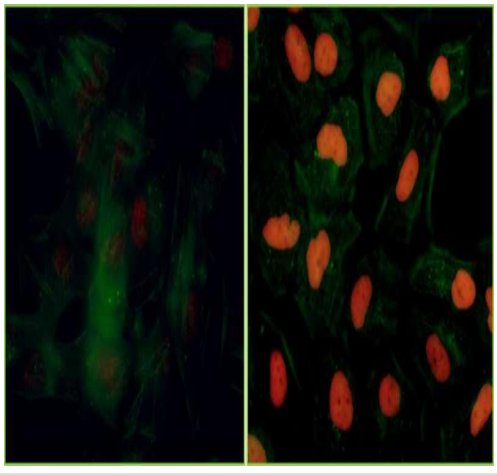
Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG antibody

Exposure time: 30 seconds



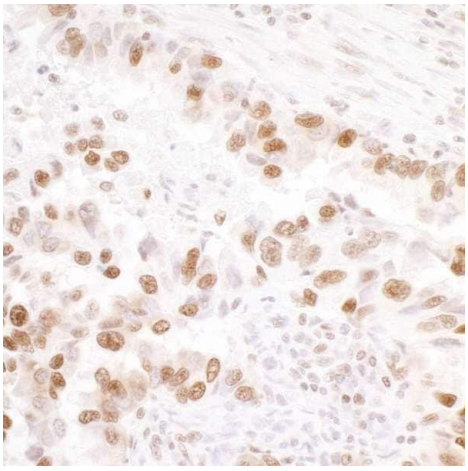
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 (phospho S824) antibody [BL-246-7B5] (ab243870)

Formalin-fixed, paraffin-embedded human breast carcinoma tissue and serial sections of etoposide treated HeLa cells stained for KAPI (phospho S824) using ab243870 at 1/100 dilution in immunohistochemical analysis. Mock phosphatase treated sections (left) and calf intestinal phosphatase-treated sections (right) are also shown. A HRP-conjugated goat anti-rabbit IgG was used as the secondary. DAB staining.



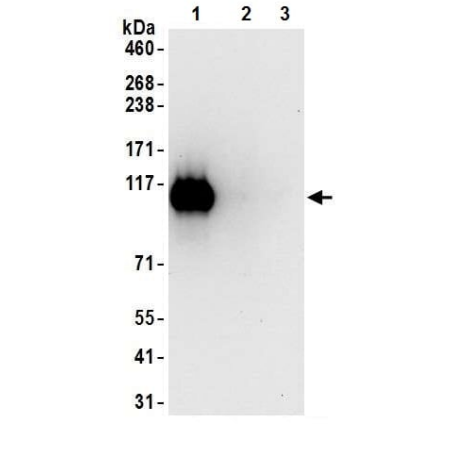
Immunocytochemistry - Anti-KAP1 (phospho S824) antibody [BL-246-7B5] (ab243870)

Formaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells treated with etoposide (right) or untreated (left) labeling KAP-1 (phospho S824) (red) using ab243862 at 1/100 dilution in ICC analysis. DyLight® 594-conjugated goat anti-rabbit IgG was used as the secondary antibody. Counterstain: Phalloidin conjugated Alexa Fluor® 488 (green).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KAP1 (phospho S824) antibody [BL-246-7B5] (ab243870)

Formalin-fixed, paraffin-embedded human lung carcinoma tissue stained for KAPI (phospho S824) using ab243870 at 1/100 dilution in immunohistochemical analysis. A HRP-conjugated goat anti-rabbit IgG was used as the secondary. DAB staining.



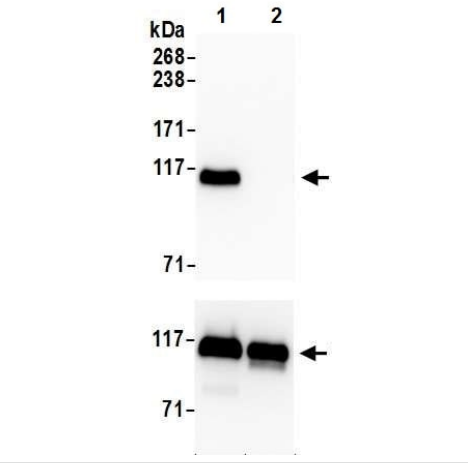
Immunoprecipitation - Anti-KAP1 (phospho S824) antibody [BL-246-7B5] (ab243870)

KAP1 (phospho S824) was immunoprecipitated from 1 mg HEK-293T whole cell lysate treated with 100 µM etoposide or untreated, using ab243870 at 20 µl per reaction. Western blot was performed on the immunoprecipitate using ab243870 at 1/1000 dilution.
Lane 1: ab243870 IP in HEK-293T cell lysate treated with 100 µM etoposide.

Lane 2: ab243870 Ip in untreated HEK-293T whole cell lysate.

Lane 3: Control IgG in HEK-293T whole cell lysate treated with 100 µM etoposide.

Detection: Chemiluminescence with an exposure time of 10 seconds



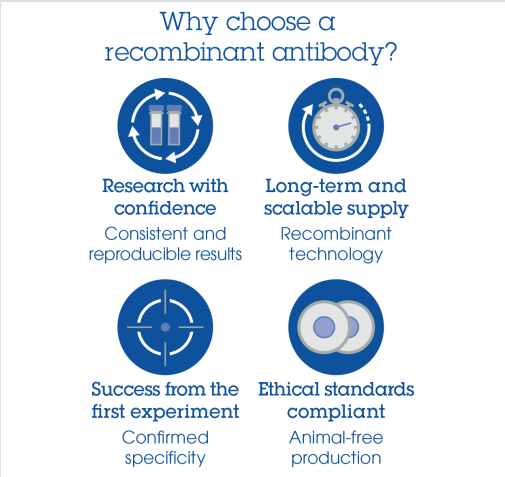
Western blot - Anti-KAP1 (phospho S824) antibody [BL-246-7B5] (ab243870)

All lanes : Anti-KAP1 (phospho S824) antibody [BL-246-7B5] (ab243870) at 1/1000 dilution

Lane 1 : HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate, treated with 100 µM etoposide
Lane 2 : HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate, mock treated

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG antibody



Anti-KAP1 (phospho S824) antibody [BL-246-7B5]

(ab243870)

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