abcam

Product datasheet

Anti-KAP1 (phospho S824) antibody ab70369

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Overview

Product name Anti-KAP1 (phospho S824) antibody

Description Rabbit polyclonal to KAP1 (phospho S824)

Host species Rabbit

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

Species reactivity Reacts with: Human

Predicted to work with: Chimpanzee, Rhesus monkey, Gorilla, Orangutan

Immunogen Synthetic peptide corresponding to Human KAP1 (phospho S824).

Database link: Q13263

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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 6.8

Preservative: 0.09% Sodium azide

Constituents: 0.1% BSA, Tris buffered saline

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab70369 in the following tested applications.

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

Application	Abreviews	Notes
ICC	**** <u>(1)</u>	Use at an assay dependent concentration.
WB	**** <u>(2)</u>	1/1000 - 1/5000. Detects a band of approximately 117 kDa (predicted molecular weight: 89 kDa).
IHC-P		Use a concentration of 0.2 µg/ml.
IP		Use at 2-5 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.

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

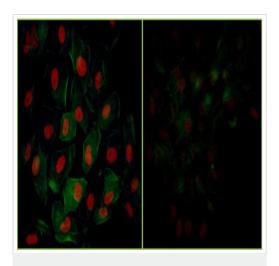
involved in cell cycle control and apoptosis in response to genotoxic stress. Dephosphorylation by the phosphatases, PPP1CA and PP1CB forms, allows sumoylation and expression of TRIM28 target genes.

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

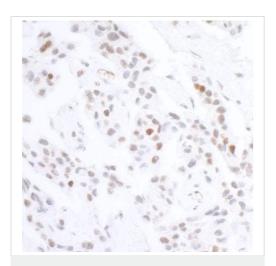
Nucleus. Associated with centromeric heterochromatin during cell differentiation through CBX1.

Images



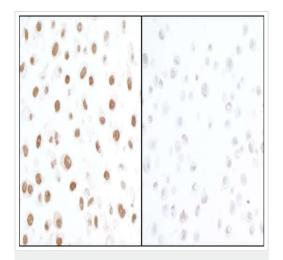
Immunocytochemistry - Anti-KAP1 (phospho S824) antibody (ab70369)

Detection of KAP1 (phospho S824) by ab70369 (1:200) in NBF-fixed HeLa cells by Immunocytochemistry. Cells were treated with etoposide (left) and untreated (right). Red fluorescent anti-rabbit IgG dylight 594 conjugated was used as a secondary antibody at 1/100 dilution.



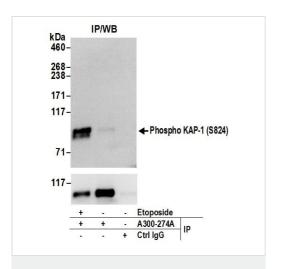
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KAP1 (phospho S824) antibody (ab70369)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human lung cancer cells labelling KAP1 with ab70369 at 0.2 μ g/mL.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-KAP1 (phospho S824) antibody (ab70369)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of HeLa cells labelling KAP1 with ab70369 at 0.2 µg/mL. Cells were treated with etoposide (left) and untreated (right).

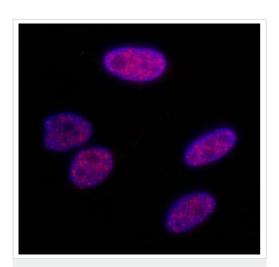


Immunoprecipitation - Anti-KAP1 (phospho S824) antibody (ab70369)

Detection of Human KAP1 (phospho S824) by Western Blot of Immunoprecipitates.

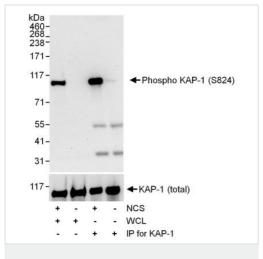
Samples: Whole cell lysate (1 mg for IP; 20% of IP loaded) from 293T cells that were mock treated (-) or treated with Etoposide (100 μ M, 2h).

Detection: Chemiluminescence with an exposure time of 30 seconds.



Immunocytochemistry - Anti-KAP1 (phospho S824) antibody (ab70369)

Detection of KAP1 (phospho S824) by ab70369 (1:200) in NBF-fixed HeLa cells by Immunocytochemistry.



Western blot - Anti-KAP1 (phospho S824) antibody (ab70369)

All lanes : Anti-KAP1 (phospho S824) antibody (ab70369) at 0.1 μ g/ml

Lane 1 : Whole cell lysate from asynchronous HeLa cells, treated with neocarzinostatin (NCS; 200 ng/ml, 30 minutes) at 50 μ g

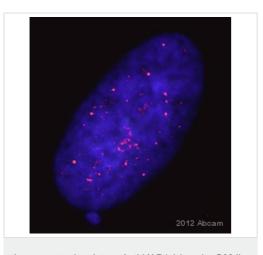
Lane 2 : Whole cell lysate from asynchronous HeLa cells at $50~\mu g$

Lane 3: Whole cell lysate from asynchronous HeLa cells, treated with neocarzinostatin (NCS; 200 ng/ml, 30 minutes) at 200 µg

Lane 4: Whole cell lysate from asynchronous HeLa cells at 200 µg

Developed using the ECL technique.

Predicted band size: 89 kDa **Observed band size:** 117 kDa



Immunocytochemistry - Anti-KAP1 (phospho S824) antibody (ab70369)

This image is courtesy of an Abreview submitted by Ms. Can Zhou $\,$

ab70369 staining KAP1 (phospho S824) (red) in Human U2OS cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were treated with 1 μ M Etoposide for 3 hours prior to fixing. Cells were fixed with formaldehyde, permeabilized in 0.5% NP40 and blocked with 3% BSA for 2 hours at 21°C. Samples were incubated with primary antibody (1/1000 in PBS + 3% BSA) for 12 hours at 4°C. An Alexa Fluor®594-conjugated Mouse anti-rabbit IgG monoclonal (1/500) was used as the secondary antibody.

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