abcam

Product datasheet

Anti-ZIP Kinase antibody [EPR18809-86] ab210528

KO VALIDATED Recombinant RabMAb

1 References 10 Images

Overview

Product name	Anti-ZIP Kinase antibody [EPR18809-86]
Description	Rabbit monoclonal [EPR18809-86] to ZIP Kinase
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human, Recombinant fragment
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human ZIP Kinase fragment recombinant protein; Human fetal liver, fetal heart, fetal kidney and bladder lysates; Jurkat, A431, HeLa, C2C12, L6, A549, C6, RAW 264.7, PC-12 and NIH/3T3 cell lysates; Mouse and rat bladder lysates; Mouse brain, kidney and spleen lysates; Rat brain, heart, kidney and spleen lysates. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): C2C12 and NIH/3T3 cells.
General notes	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**.

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: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR18809-86
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab210528 in the following tested applications.

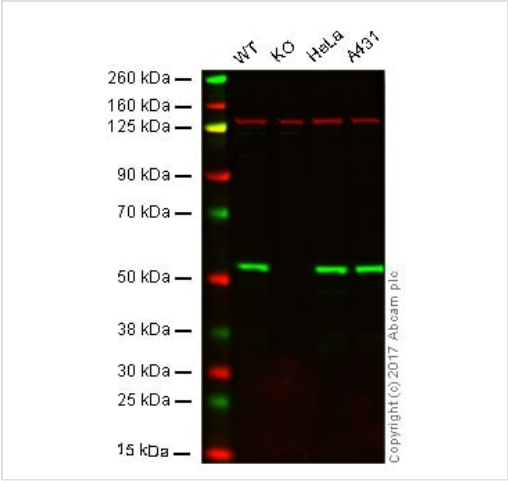
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/120.
WB		1/1000. Detects a band of approximately 50 kDa (predicted molecular weight: 53 kDa).
ICC/IF		1/100.

Target

Function	Serine/threonine kinase which acts as a positive regulator of apoptosis. Phosphorylates histone H3 on 'Thr-11' at centromeres during mitosis. Regulates myosin light chain phosphatase through phosphorylation of MYPT1 thereby regulating the assembly of the actin cytoskeleton, cell migration, invasiveness of tumor cells, smooth muscle contraction and neurite outgrowth. Involved in the formation of promyelocytic leukemia protein nuclear body (PML-NB), one of many subnuclear domains in the eukaryotic cell nucleus, and which is involved in oncogenesis and viral infection.
Sequence similarities	Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. DAP kinase subfamily. Contains 1 protein kinase domain.
Post-translational modifications	Ubiquitinated. Ubiquitination mediated by the UBE2D3 E3 ligase does not lead to proteasomal degradation, but influences promyelocytic leukemia protein nuclear bodies (PML-NBs) formation in the nucleus. Autophosphorylated. Phosphorylated by ROCK1.
Cellular localization	Nucleus. Cytoplasm. Nucleus > PML body. Relocates to the cytoplasm on binding PAWR where the complex appears to interact with actin filaments (By similarity). Localizes to promyelocytic leukemia protein nuclear bodies (PML-NBs). Associates to centromeres from prophase to anaphase.

Images

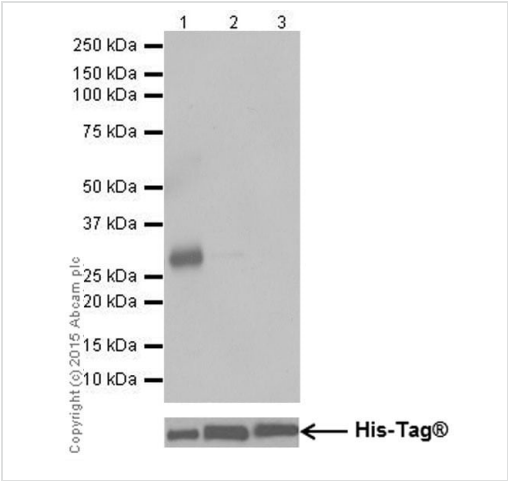


Western blot - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: ZIP Kinase knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: A431 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab210528 observed at 53 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab210528 was shown to specifically react with ZIP Kinase in wild type cells as signal was lost in ZIP Kinase knockout cells. Wild-type and ZIP Kinase knockout samples were subjected to SDS-PAGE. Ab210528 and **ab9484** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at a 1/1000 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 - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

All lanes : Anti-ZIP Kinase antibody [EPR18809-86] (ab210528) at 1/1000 dilution

Lane 1 : Human ZIP Kinase fragment recombinant protein
Lane 2 : Human DAP Kinase 2 fragment recombinant protein
Lane 3 : Human DAP Kinase 1 fragment recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

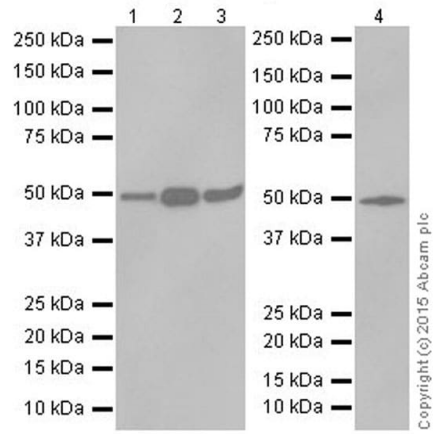
Predicted band size: 53 kDa
Observed band size: 29 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

Human ZIP Kinase fragment recombinant protein contain aa13-275 with a His-Tag®. Human DAP Kinase 2 fragment recombinant protein contain aa23-285 with a His-Tag®. Human DAP Kinase 1 fragment recombinant protein contain aa13-275 with a His-Tag®. All three recombinant human fragment proteins were made in-house.

The antibody reacts weakly with DAP kinase 1 and DAP kinase 2; however bands of their appropriate MW (159 kD, 42 kD) are not detected in the tissue lysates.



Western blot - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

All lanes : Anti-ZIP Kinase antibody [EPR18809-86] (ab210528) at 1/1000 dilution

Lane 1 : Human fetal liver lysate
Lane 2 : Human fetal heart lysate
Lane 3 : Human fetal kidney lysate
Lane 4 : Human bladder lysate

Lysates/proteins at 10 µg per lane.

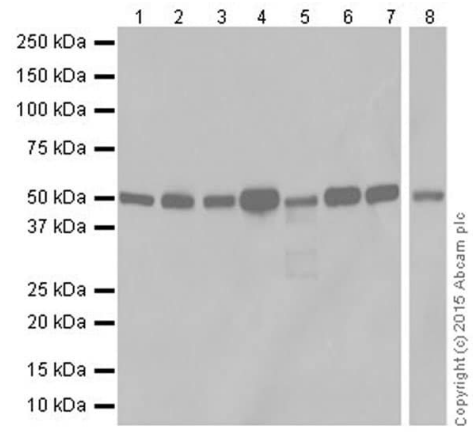
Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 53 kDa
Observed band size: 50 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID: 20124481).



Western blot - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

All lanes : Anti-ZIP Kinase antibody [EPR18809-86] (ab210528) at 1/1000 dilution

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 2 : A431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : C2C12 (Mouse myoblast cell line) whole cell lysate
Lane 5 : L6 (Rat skeletal muscle cell line) whole cell lysate
Lane 6 : Mouse bladder lysate
Lane 7 : Rat bladder lysate
Lane 8 : A549 (Human lung carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

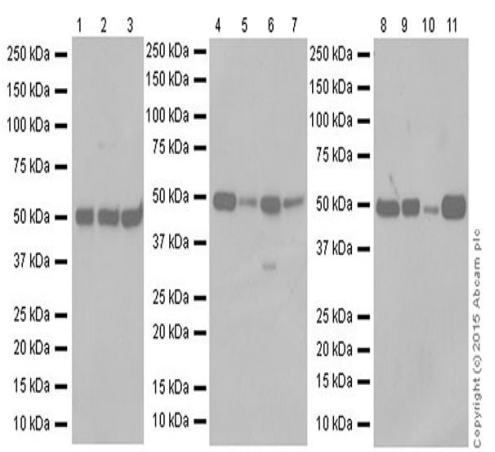
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 53 kDa
Observed band size: 50 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1, 2, 3, 4, 5, 6 and 7: 30 seconds; Lane 8: 5 seconds.

The molecular weight observed is consistent with what has been described in the literature (PMID: 20124481).



Western blot - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

All lanes : Anti-ZIP Kinase antibody [EPR18809-86] (ab210528) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate at 10 µg
Lane 2 : Mouse kidney tissue lysate at 10 µg
Lane 3 : Mouse spleen tissue lysate at 10 µg
Lane 4 : Rat brain tissue lysate at 10 µg
Lane 5 : Rat heart tissue lysate at 10 µg
Lane 6 : Rat kidney tissue lysate at 10 µg
Lane 7 : Rat spleen tissue lysate at 10 µg
Lane 8 : C6 (Rat glial tumor cell line) whole cell lysate at 20 µg
Lane 9 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 10 µg
Lane 10 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate at 10 µg
Lane 11 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate at 10 µg

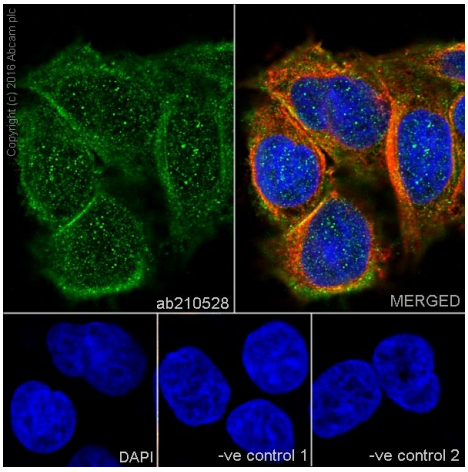
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 53 kDa
Observed band size: 50 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1, 2 and 3: 30 seconds; Lane 4, 5, 6 and 7: 3 minutes; Lane 8, 9, 10 and 11: 30 seconds.

The molecular weight observed is consistent with what has been described in the literature (PMID: 20124481)



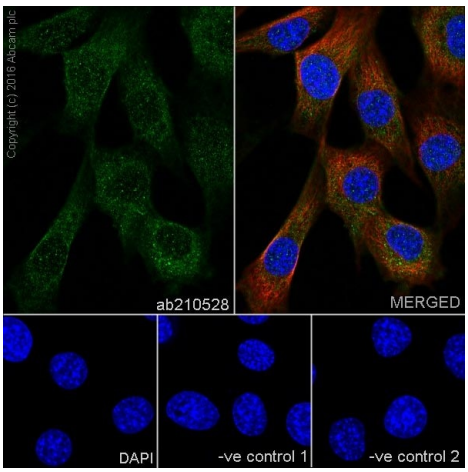
Immunocytochemistry/ Immunofluorescence - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling ZIP Kinase with ab210528 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasm and nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab210528 at 1/100 dilution followed by **ab150120** at 1/1000 dilution.
-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling ZIP Kinase with ab210528 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasm and nuclear staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

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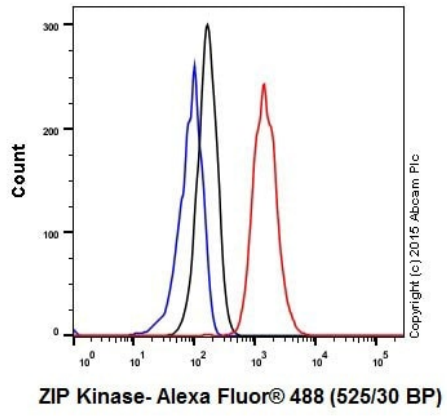
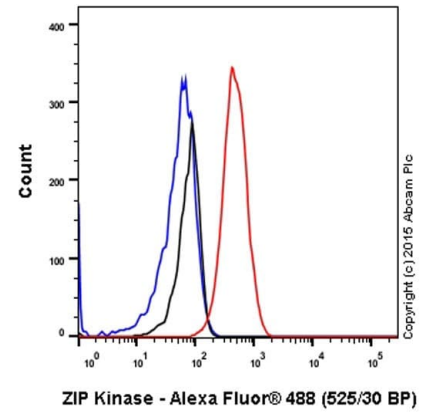
-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed C2C12 (Mouse myoblast cell line) cells labeling ZIP Kinase with ab210528 at 1/120 dilution (red) compared with a Rabbit IgG,monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/500 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling ZIP Kinase with ab210528 at 1/600 dilution (red) compared with a Rabbit IgG,monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/500 dilution was used as the secondary antibody.

Note:Cells were permeabilised with 90% methanol -1XPBS (-20?, 30min).

Anti-ZIP Kinase antibody [EPR18809-86] (ab210528)

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