abcam

Product datasheet

Anti-ERK5 antibody [EP791Y] ab40809

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-ERK5 antibody [EP791Y]
Description	Rabbit monoclonal [EP791Y] to ERK5
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human ERK5 aa 800-900 (C terminal). The exact sequence is proprietary.
Positive control	WB: HeLa, HAP1, NIH/3T3 and PC-12 cell lysates. Flow Cyt (intra): HeLa and A549 cells. ICC: HeLa cells. IP: HeLa cell lysate.
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. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.**

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: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP791Y
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab40809 in the following tested applications.

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

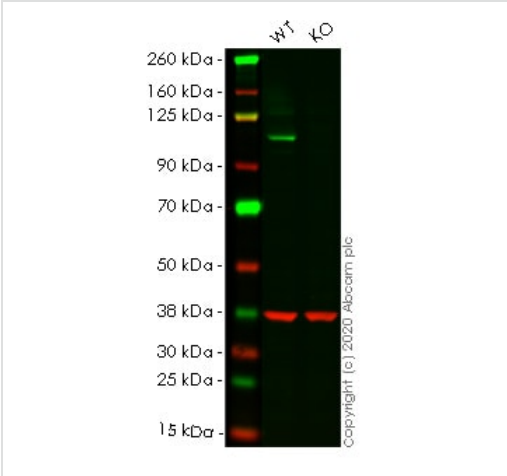
Application	Abreviews	Notes
Flow Cyt (Intra)		1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100 dilution.
WB	★★★★★ (1)	1/10000. Detects a band of approximately 115 kDa (predicted molecular weight: 89 kDa). **For unpurified use at 1/1000 - 1/5000 dilution.**
IP		1/30. **For unpurified use at 1/50 dilution.**
ICC/IF		1/100. **For unpurified use at 1/250 - 1/500 dilution.**

Target

Function	Plays a role in various cellular processes such as proliferation, differentiation and cell survival. The upstream activator of MAPK7 is the MAPK kinase MAP2K5. Upon activation, it translocates to the nucleus and phosphorylates various downstream targets including MEF2C. EGF activates MAPK7 through a Ras-independent and MAP2K5-dependent pathway. May have a role in muscle cell differentiation. May be important for endothelial function and maintenance of blood vessel integrity. MAP2K5 and MAPK7 interact specifically with one another and not with MEK1/ERK1 or MEK2/ERK2 pathways.
Tissue specificity	Expressed in many adult tissues. Abundant in heart, placenta, lung, kidney and skeletal muscle. Not detectable in liver.
Sequence similarities	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily. Contains 1 protein kinase domain.
Domain	The second proline-rich region may interact with actin targeting the kinase to a specific location in the cell. The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.
Post-translational modifications	Dually phosphorylated on Thr-219 and Tyr-221, which activates the enzyme (By similarity). Autophosphorylated in vitro on threonine and tyrosine residues when the C-terminal part of the kinase, which could have a regulatory role, is absent.

Cellular localization	Cytoplasm. Nucleus. Translocates to the nucleus upon activation.

Images



Western blot - Anti-ERK5 antibody [EP791Y] (ab40809)

All lanes : Anti-ERK5 antibody [EP791Y] (ab40809) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MAPK7 knockout HeLa cell lysate

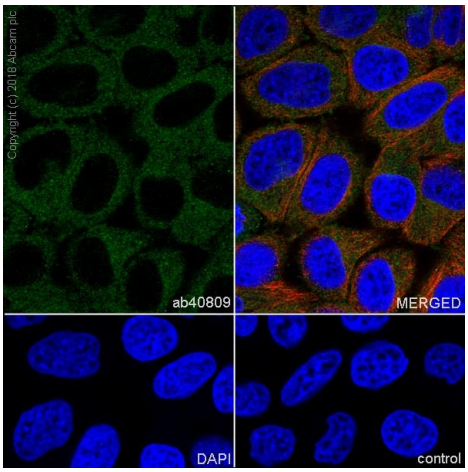
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 89 kDa
Observed band size: 115 kDa

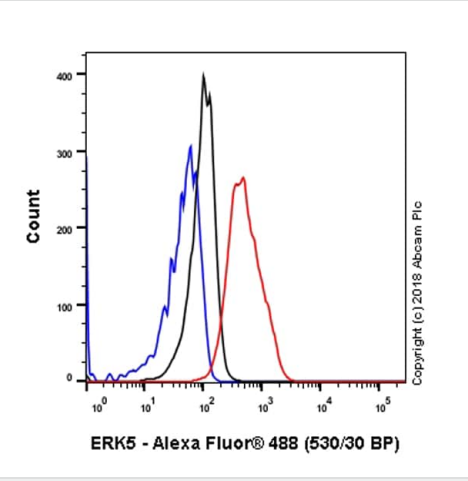
Lanes 1- 2: Merged signal (red and green). Green - ab40809 observed at 115 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab40809 was shown to react with ERK5 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265508 (knockout cell lysate ab258042) was used. Wild-type HeLa and MAPK7 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab40809 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



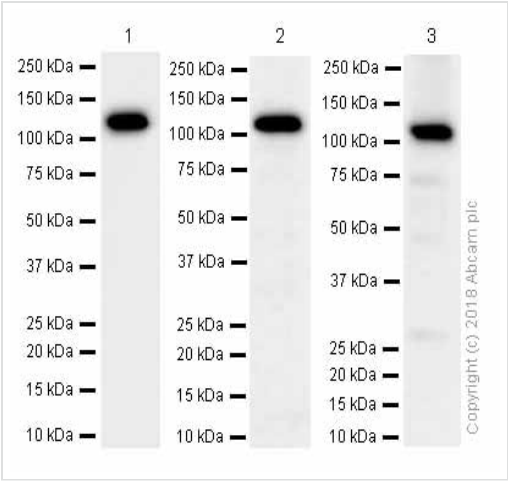
Immunocytochemistry/ Immunofluorescence - Anti-ERK5 antibody [EP791Y] (ab40809)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling ERK5 with Purified ab40809 at 1:100 (4.8 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-ERK5 antibody [EP791Y] (ab40809)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling ERK5 with purified ab40809 at 1/50 dilution (10 ug/ml) (red). Cells were fixed with 80% methanol. A Goat anti rabbit IgG (Alexa Fluorr®488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-ERK5 antibody [EP791Y] (ab40809)

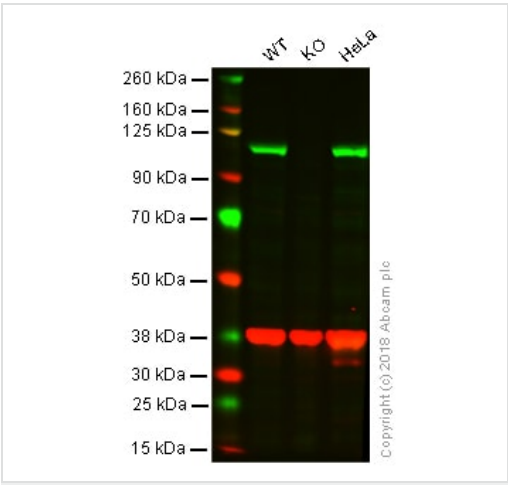
All lanes : Anti-ERK5 antibody [EP791Y] (ab40809) at 1/2000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates with 5% NFDM/TBST
Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates with 5% NFDM/TBST

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 89 kDa
Observed band size: 115 kDa

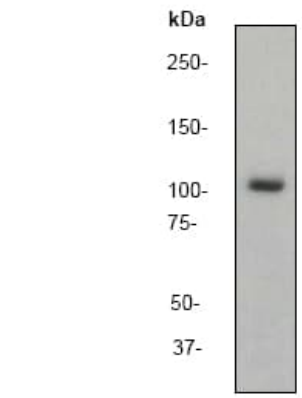


Western blot - Anti-ERK5 antibody [EP791Y] (ab40809)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: MAPK7 (ERK5) knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)

Lanes 1 - 3: Merged signal (red and green). Green - ab40809 observed at 88 kDa. Red - loading control, ab9484, observed at 37 kDa.

Unpurified ab40809 was shown to specifically react with ERK5 in wild-type HAP1 cells as signal was lost in MAPK7 (ERK5) knockout cells. Wild-type and MAPK7 (ERK5) knockout samples were subjected to SDS-PAGE. Unpurified ab40809 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 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.

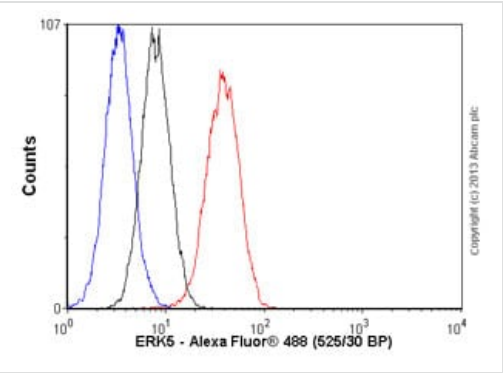


Western blot - Anti-ERK5 antibody [EP791Y] (ab40809)

Anti-ERK5 antibody [EP791Y] (ab40809) at 1/5000 dilution (unpurified) + Hela cell lysate at 10 µg

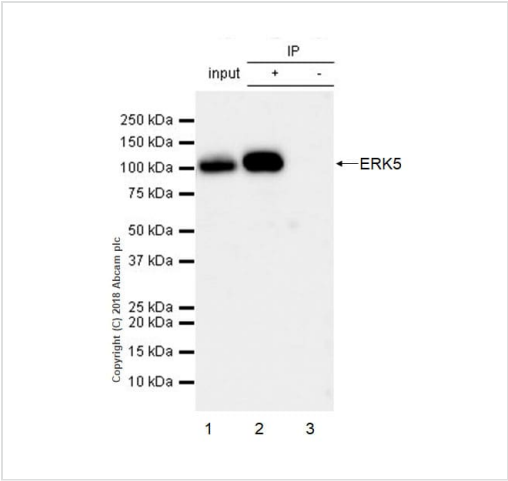
Predicted band size: 89 kDa
Observed band size: 115 kDa

The predicted weight of 89 kDa is for the precursor version of human ERK5 protein. However, ab40809 detects endogenous levels of total Erk5 protein which appears around 115 kDa in SDS PAGE



Flow Cytometry (Intracellular) - Anti-ERK5 antibody [EP791Y] (ab40809)

Overlay histogram showing A549 cells stained with unpurified ab40809 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40809, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Immunoprecipitation - Anti-ERK5 antibody [EP791Y] (ab40809)

ab40809 (purified) at 1:30 dilution (2ug) immunoprecipitating ERK5 in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

Lane 2 (+): ab40809 & HeLa whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab40809 in HeLa whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

Anti-ERK5 antibody [EP791Y] (ab40809)

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