abcam

Product datasheet

Anti-ERK5 antibody [EP791Y] - BSA and Azide free ab232538

敲除验证 重组 RabMAb

7 图像

概述

产品名称	Anti-ERK5抗体[EP791Y] - BSA and Azide free
描述	兔单克隆抗体[EP791Y] to ERK5 - BSA and Azide free
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IP, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HAP1 and HeLa whole cell lysate. IP: HeLa cell lysate. Flow Cyt (intra): HeLa cells. ICC: HeLa cells.
常规说明	ab232538 is the carrier-free version of **ab40809**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.2 Constituent: PBS
无载体	是
纯度	Protein A purified
克隆	单克隆
克隆编号	EP791Y
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab232538于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 115 kDa (predicted molecular weight: 88 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

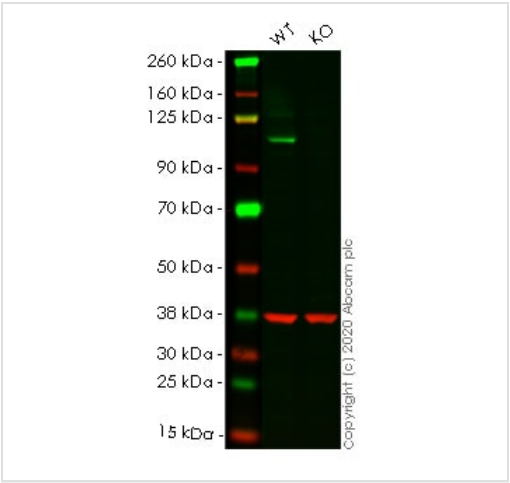
靶标

功能	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.
组织特异性	Expressed in many adult tissues. Abundant in heart, placenta, lung, kidney and skeletal muscle. Not detectable in liver.
序列相似性	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily. Contains 1 protein kinase domain.
结构域	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.

翻译后修饰	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.
细胞定位	Cytoplasm. Nucleus. Translocates to the nucleus upon activation.

图片



Western blot - Anti-ERK5 antibody [EP791Y] - BSA and Azide free (ab232538)

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.

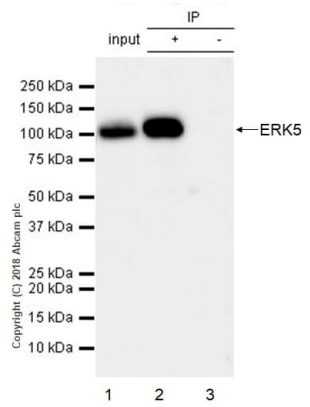
Performed under reducing conditions.

Predicted band size: 88 kDa
Observed band size: 115 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab40809**).

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.



Immunoprecipitation - Anti-ERK5 antibody [EP791Y] - BSA and Azide free (ab232538)

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

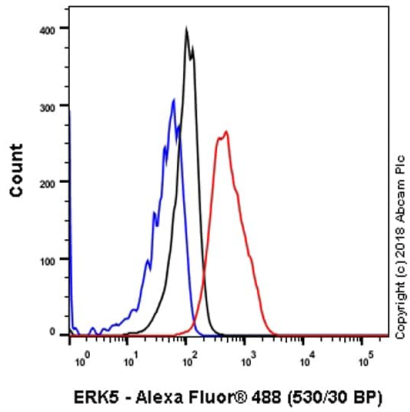
Lane 2 (+): ab40809 & HeLa whole cell lysate

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.

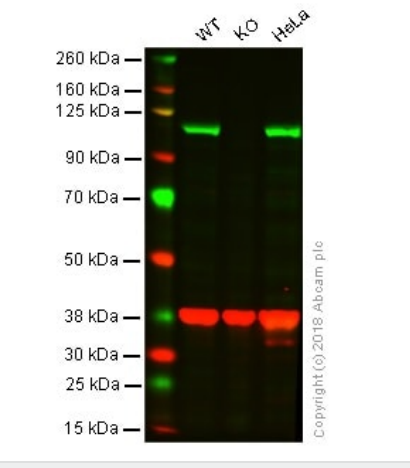
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40809**).



Flow Cytometry (Intracellular) - Anti-ERK5 antibody [EP791Y] - BSA and Azide free (ab232538)

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 Fluor® 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40809**).



Western blot - Anti-ERK5 antibody [EP791Y] - BSA and Azide free (ab232538)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: MAPK7 (ERK5) knockout HAP1 whole cell lysate (20 µg)

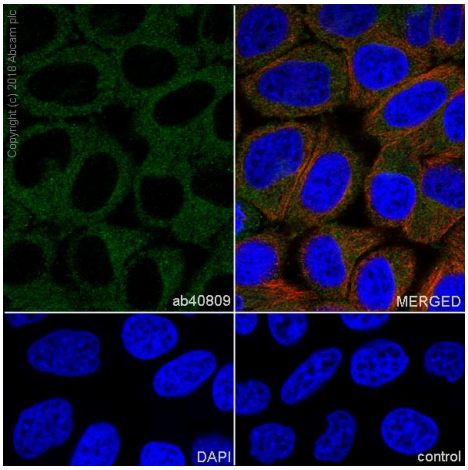
Lane 3: HeLa whole cell lysate (20 µg)

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

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. 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.

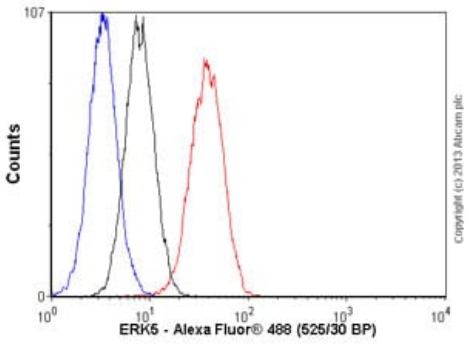
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40809**).



Immunocytochemistry/ Immunofluorescence - Anti-ERK5 antibody [EP791Y] - BSA and Azide free (ab232538)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40809**).



Flow Cytometry (Intracellular) - Anti-ERK5 antibody [EP791Y] - BSA and Azide free (ab232538)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab40809**).

Anti-ERK5 antibody [EP791Y] - BSA and Azide free

(ab232538)

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