abcam

Product datasheet

Anti-ERK1 antibody [EP4967] ab109282

KO VALIDATED Recombinant RabMAb

7 References 12 Images

Overview

Product name	Anti-ERK1 antibody [EP4967]
Description	Rabbit monoclonal [EP4967] to ERK1
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T, Jurkat, A375, A431 and HUVEC cell lysates. IHC-P: Human colon adenocarcinoma tissue. Flow Cyt (intra): Jurkat cells, Hap1 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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP4967
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109282 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)		1/30. **ab172730**-Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000 - 1/10000. Predicted molecular weight: 44 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. Heat up to 98°C, below boiling, and then let cool for 10-20 min.
ICC/IF		1/100.

Target

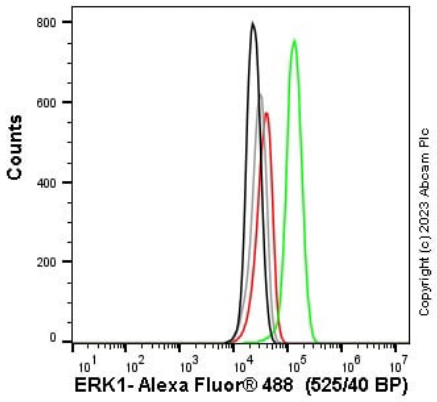
Function Involved in both the initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells by phosphorylating a number of transcription factors such as ELK-1. Phosphorylates EIF4EBP1; required for initiation of translation. Phosphorylates microtubule-associated protein 2 (MAP2). Phosphorylates SPZ1 (By similarity). Phosphorylates heat shock factor protein 4 (HSF4).

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily.
Contains 1 protein kinase domain.

Domain The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.

Post-translational modifications Dually phosphorylated on Thr-202 and Tyr-204, which activates the enzyme. Dephosphorylated by PTPRJ at Tyr-204.

Images



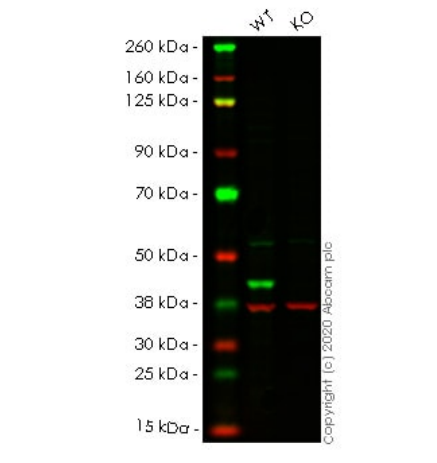
Flow Cytometry (Intracellular) - Anti-ERK1 antibody [EP4967] (ab109282)

Flow cytometry overlay histogram showing wild-type Hap1 (green line) and MAPK3 knockout Hap1 stained with ab109282 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab109282) (1x 10^6 in 100µl at 0.2 µg/ml (1/11400)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, MAPK3 knockout Hap1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-ERK1 antibody [EP4967] (ab109282)

All lanes : Anti-ERK1 antibody [EP4967] (ab109282) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : MAPK3 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

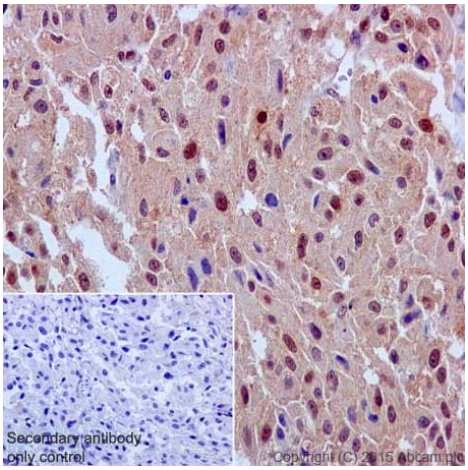
Performed under reducing conditions.

Predicted band size: 44 kDa
Observed band size: 43 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab109282 observed at 43 kDa. Red - Anti-GAPDH antibody [6C5] - Loading

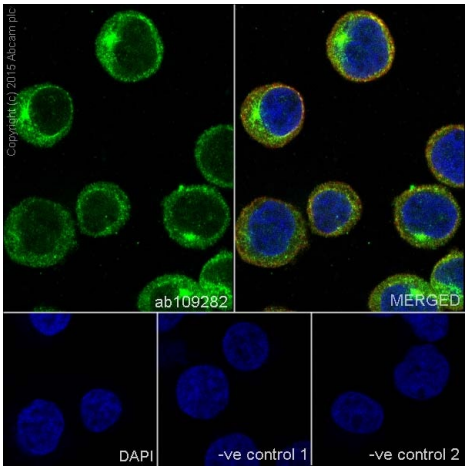
Control (**ab8245**) observed at 37 kDa.

ab109282 was shown to react with ERK1 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266519** (knockout cell lysate **ab257099**) was used. Wild-type HEK-293T and MAPK3 knockout HEK-293T 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. ab109282 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [EP4967] (ab109282)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human melanoma tissue labelling ERK1 with purified ab109282 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a goat anti-rabbit IgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

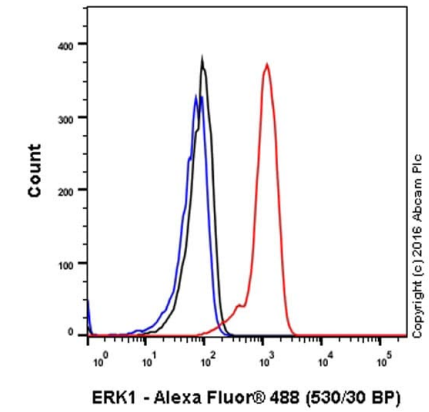


Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [EP4967] (ab109282)

Immunocytochemistry/Immunofluorescence analysis of Jurkat (human acute T cell leukemia) cells labelling ERK1 with purified ab109282 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

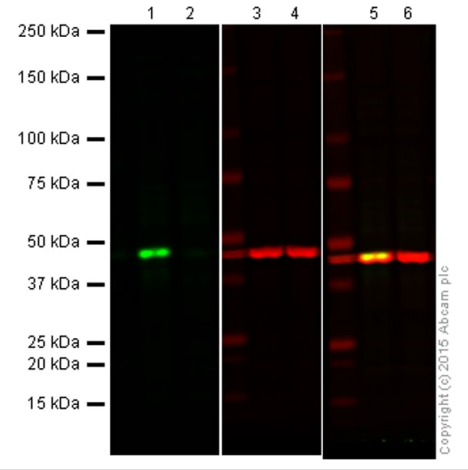
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [EP4967] (ab109282)

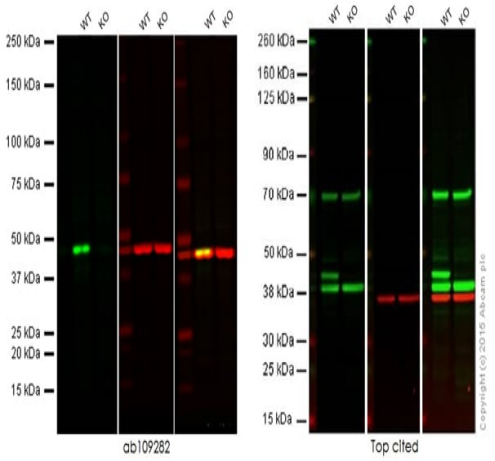
Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells labeling ERK1 with purified ab109282 at 1/30 dilution (red). The secondary antibody was Goat anti rabbit IgG (Alexa Fluorr® 488) at 1/2000 dilution. A Rabbit monoclonal IgG (Black) was used as the isotype control and cells without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.



Western blot - Anti-ERK1 antibody [EP4967] (ab109282)

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: ERK1 knockout HAP1 cell lysate (20 µg)
Lanes 1 and 2: Green signal from target - ab109282 observed at 42 kDa
Lanes 3 and 4: Red signal from loading control - **ab8226** observed at 42 kDa
Lanes 5 and 6: Merged (red and green) signal
ab109282 was shown to specifically react with ERK1 when ERK1 knockout samples were used.
Wild-type and ERK1 knockout samples were subjected to SDS-PAGE. ab109282 and **ab8226** (loading control to beta actin) were both diluted 1/1000 and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



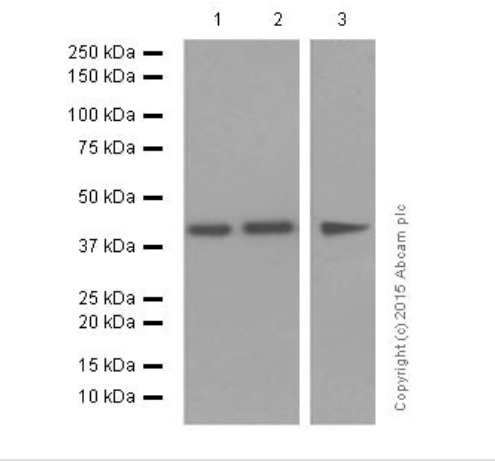
Western blot - Anti-ERK1 antibody [EP4967] (ab109282)

Predicted band size : 43 kDa

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: ERK1 knockout HAP1 cell lysate (20 µg)
Lanes 1 and 2: Green signal from target
Lanes 3 and 4: Red signal from loading control
Lanes 5 and 6: Merged (red and green) signal

Red - loading control, **ab8226** observed at 42 kDa or **ab8245**, observed at 37 kDa

This western blot image is a comparison between ab109282 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-ERK1 antibody [EP4967] (ab109282)

All lanes : Anti-ERK1 antibody [EP4967] (ab109282) at 1/10000 dilution (purified)

Lane 1 : Mouse brain lysate
Lane 2 : Rat brain lysate
Lane 3 : NIH/3T3 cell lysate

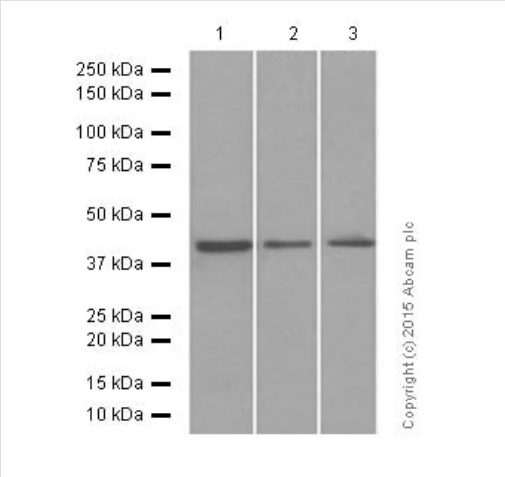
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 44 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-ERK1 antibody [EP4967] (ab109282)

All lanes : Anti-ERK1 antibody [EP4967] (ab109282) at 1/10000 dilution (purified)

Lane 1 : Jurkat cell lysate
Lane 2 : A375 cell lysate
Lane 3 : HUVEC cell lysate

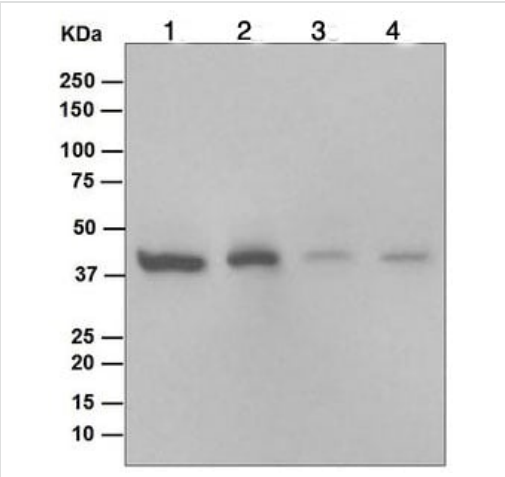
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 44 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



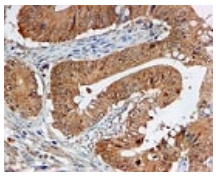
Western blot - Anti-ERK1 antibody [EP4967] (ab109282)

All lanes : Anti-ERK1 antibody [EP4967] (ab109282) at 1/1000 dilution (unpurified)

Lane 1 : Jurkat cell lysate
Lane 2 : A375 cell lysate
Lane 3 : A431 cell lysate
Lane 4 : HUVEC cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 44 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [EP4967] (ab109282)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colonic adenocarcinoma tissue labelling ERK1 with unpurified ab109282 at 1/100 dilution.

Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.

Anti-ERK1 antibody [EP4967] (ab109282)

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