abcam

Product datasheet

Anti-ERK1 + ERK2 antibody [EPR17526] ab184699

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-ERK1 + ERK2 antibody [EPR17526]
Description	Rabbit monoclonal [EPR17526] to ERK1 + ERK2
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), IP, ICC/IF, WB
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human ERK1 full length recombinant protein; Human ERK2 full length recombinant protein; A431, Jurkat, HeLa, HepG2, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Human fetal brain, fetal heart and fetal kidney lysates; Mouse brain, heart, kidney and spleen lysates; Rat brain, heart, kidney and spleen lysates. ICC/IF: HeLa cells. Flow Cyt (intra): A431 cells. IP. PC-12 whole cell extract.
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, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR17526
lsotype	lgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab184699 in the following tested applications.

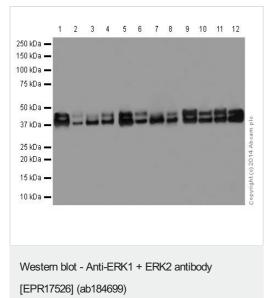
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/440. <u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		1/70.
ICC/IF	★ ★ ★ ★ ★ (1)	1/250.
WB		1/10000. Detects a band of approximately 44, 42 kDa (predicted molecular weight: 43, 41 kDa).

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 ELK1. Phosphorylates EIF4EBP1; required for initiation of translation. Phosphorylates microtubule- associated protein 2 (MAP2). Phosphorylates SPZ1 (By similarity). Phosphorylates heat shock factor protein 4 (HSF4) and ARHGEF2. Acts as a transcriptional repressor. Binds to a [GC]AAA[GC] consensus sequence. Repress the expression of interferon gamma-induced genes. Seems to bind to the promoter of CCL5, DMP1, IFIH1, IFITM1, IRF7, IRF9, LAMP3, OAS1, OAS2, OAS3 and STAT1. Transcriptional activity is independent of kinase activity.
Sequence similarities	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily. 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-185 and Tyr-187, which activates the enzyme. Dephosphorylated by PTPRJ at Tyr-187.
Cellular localization	Nucleus.
Form	Mainly expressed in the cytoplasm and only localizes to the nucleus with treatment.

Images



All lanes : Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) at 1/10000 dilution

Lane 1 : Mouse brain lysates

- Lane 2 : Mouse heart lysates
- Lane 3 : Mouse kidney lysates
- Lane 4 : Mouse spleen lysates
- Lane 5 : Rat brain lysates
- Lane 6 : Rat heart lysates
- Lane 7 : Rat kidney lysates
- Lane 8 : Rat spleen lysates
- Lane 9: C6 (Rat glial tumor cells) whole cell lysates

Lane 10 : RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) whole cell lysates

Lane 11 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates

Lane 12 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysates

Lysates/proteins at 10 µg per lane.

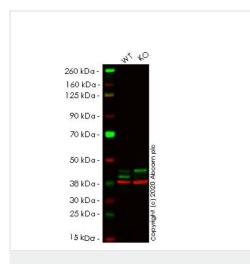
Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 43, 41 kDa **Observed band size:** 44, 42 kDa

44kDa band represents ERK1. 42kDa band represents ERK2.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) All lanes : Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate Lane 2 : MAPK1 knockout HeLa cell lysate

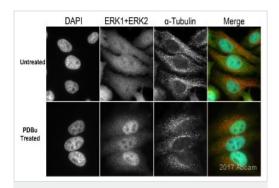
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 43, 41 kDa Observed band size: 44 kDa

Lanes 1-2: Merged signal (red and green). Green - ab184699 observed at 44 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

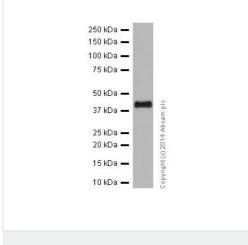
ab184699 Anti-ERK1 + ERK2 antibody [EPR17526] was shown to specifically react with ERK2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line <u>ab265052</u> (knockout cell lysate <u>ab257525</u>) was used. Wild-type and ERK2 knockout samples were subjected to SDS-PAGE. ab184699 and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at 1 in 10000 Dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye[®] 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Ab184699 staining ERK1 + ERK2 in HeLa cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100. Samples were incubated with primary antibody (1/500 in PBS) for 1 hour at 22°C. A Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed (**ab150081**) was used as the secondary antibody.

Immunocytochemistry/ Immunofluorescence - Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699)

This image is courtesy of an Abreview submitted by Kirk Mcmanus.



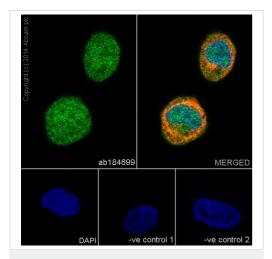
Western blot - Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) at 1/10000 dilution + Recombinant Human ERK1 protein (<u>ab43623</u>) (<u>ab43623</u>) at 0.01 μg

Secondary

Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 43, 41 kDa Observed band size: 44 kDa

Recombinant full length ERK1 protein (<u>ab43623</u>) contains aa1-379. Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699)

> 250 kDa — 150 kDa — 100 kDa — 75 kDa —

> > 50 kDa — 37 kDa —

25 kDa — 20 kDa —

15 kDa 🗕

10 kDa 🗕

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling ERK1 + ERK2 with ab184699 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Confocal image showing both nuclear and cytoplasmic staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab184699 at 1/250 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.

Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) at 1/10000 dilution + Recombinant Human ERK2 protein (**ab43625**) at 0.01 µg

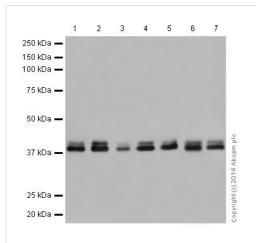
Secondary

Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 43, 41 kDa Observed band size: 42 kDa



Recombinant full length ERK2 protein (<u>ab43625</u>) contains aa1-360. Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) All lanes : Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) at 1/50000 dilution

Lane 1 : A431 (Human epidermoid carcinoma) whole cell lysates Lane 2 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysates

Lane 3 : HeLa (Human epithelial cells from cervix

adenocarcinoma) whole cell lysates

Lane 4 : HepG2 (Human liver hepatocellular carcinoma) whole cell lysates

Lane 5 : Human fetal brain lysates

Lane 6 : Human fetal heart lysates

Lane 7 : Human fetal kidney lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 43, 41 kDa **Observed band size:** 42,44 kDa

44kDa band represents ERK1. 42kDa band represents ERK2. Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes : Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) at 1/10000 dilution

Lane 1 : Human fetal brain lysates Lane 2 : Human fetal heart lysates

Lysates/proteins at 10 µg per lane.

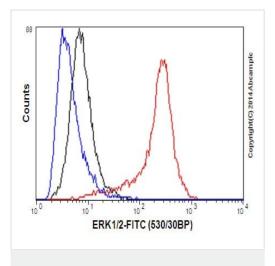
Secondary

All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 43, 41 kDa Observed band size: 42,44 kDa

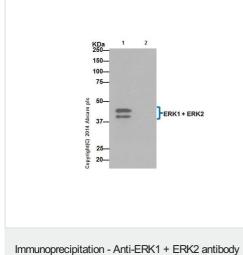


Western blot - Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699) 44kDa band represents ERK1. 42kDa band represents ERK2. Blocking/Dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-ERK1 + ERK2 antibody [EPR17526] (ab184699)

Intracellular flow cytometric analysis of A431 (Human epidermoid carcinoma) cells labeling ERK1 + ERK2 with ab184699 at 1/440 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.



[EPR17526] (ab184699)

ERK1 + ERK2 were immunoprecipitated from 1mg of PC-12 (Rat adrenal gland pheochromocytoma) whole cell extract with ab184699 at 1/70 dilution. Western blot was performed from the immunoprecipitate using ab184699 at 1/5000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: PC-12 whole cell extract. Lane 2: PBS instead of PC-12 whole cell extract.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

44kDa band represents ERK1. 42kDa band represents ERK2.



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