abcam

Product datasheet

Anti-MEK1 + MEK2 antibody [EPR16667] ab178876

Recombinant RabMAb

★★★★★ 1 Abreviews 47 References 11 Images

Overview

Product name	Anti-MEK1 + MEK2 antibody [EPR16667]
Description	Rabbit monoclonal [EPR16667] to MEK1 + MEK2
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Jurkat, Daudi, HeLa, 293T, A549 and A431 whole cell lysates; Human fetal brain, heart, kidney and spleen lysates; Mouse and Rat brain and heart lysates. IHC-P: Human renal medulla, Mouse lung and Rat lung tissues. ICC/IF: NIH/3T3 cells. IP: HeLa 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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16667
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab178876 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
WB		1/20000. Detects a band of approximately 44 kDa (predicted molecular weight: 43, 44 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (1)	1/100.
IP		1/120.
Flow Cyt (Intra)		Use at an assay dependent concentration. Purified format.

Target

Function Dual specificity protein kinase which acts as an essential component of the MAP kinase signal transduction pathway. Binding of extracellular ligands such as growth factors, cytokines and hormones to their cell-surface receptors activates RAS and this initiates RAF1 activation. RAF1 then further activates the dual-specificity protein kinases MAP2K1/MEK1 and MAP2K2/MEK2. Both MAP2K1/MEK1 and MAP2K2/MEK2 function specifically in the MAPK/ERK cascade, and catalyze the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in the extracellular signal-regulated kinases MAPK3/ERK1 and MAPK1/ERK2, leading to their activation and further transduction of the signal within the MAPK/ERK cascade. Depending on the cellular context, this pathway mediates diverse biological functions such as cell growth, adhesion, survival and differentiation, predominantly through the regulation of transcription, metabolism and cytoskeletal rearrangements. One target of the MAPK/ERK cascade is peroxisome proliferator-activated receptor gamma (PPARG), a nuclear receptor that promotes differentiation and apoptosis. MAP2K1/MEK1 has been shown to export PPARG from the nucleus. The MAPK/ERK cascade is also involved in the regulation of endosomal dynamics, including lysosome processing and endosome cycling through the perinuclear recycling compartment (PNRC), as well as in the fragmentation of the Golgi apparatus during mitosis.

Tissue specificity Widely expressed, with extremely low levels in brain.

Involvement in disease Cardiofaciocutaneous syndrome 3

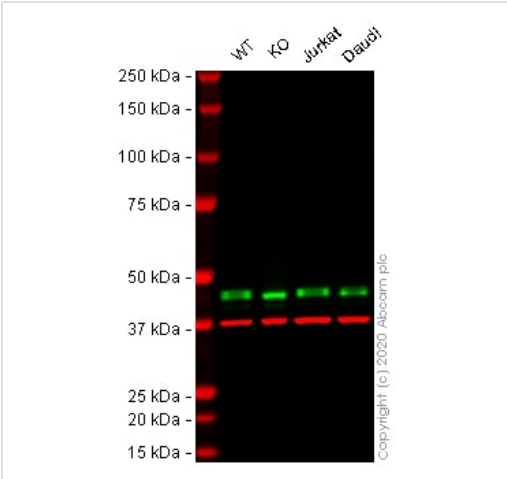
Sequence similarities Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase subfamily.
Contains 1 protein kinase domain.

Domain The proline-rich region localized between residues 270 and 307 is important for binding to RAF1 and activation of MAP2K1/MEK1.

Post-translational modifications Phosphorylation at Ser-218 and Ser-222 by MAP kinase kinase kinases (RAF or MEKK1) positively regulates kinase activity. Also phosphorylated at Thr-292 by MAPK1/ERK2 and at Ser-298 by PAK. MAPK1/ERK2 phosphorylation of Thr-292 occurs in response to cellular adhesion and leads to inhibition of Ser-298 phosphorylation by PAK.

	Acetylation by Yersinia yopJ prevents phosphorylation and activation, thus blocking the MAPK signaling pathway.
Cellular localization	Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Cytoplasm, cytoskeleton, microtubule organizing center, spindle pole body. Cytoplasm. Nucleus. Localizes at centrosomes during prometaphase, midzone during anaphase and midbody during telophase/cytokinesis.

Images



Western blot - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

All lanes : Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876) at 1/20000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : MAP2K2 knockout HEK293T cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Daudi cell lysate

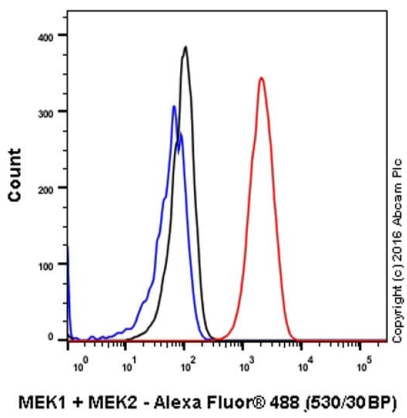
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 43, 44 kDa
Observed band size: 45 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab178876 observed at 45 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab178876 was shown to react with MEK2 in wild-type HEK-293T cells in western blot with loss of signal observed in MAP2K2 knockout sample. Wild-type HEK-293T and MAP2K2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab178876 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 20000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.

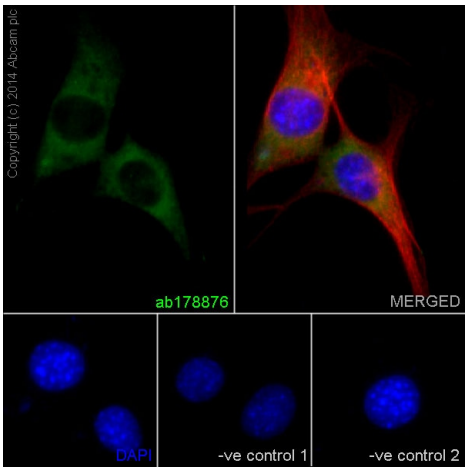


Flow Cytometry (Intracellular) - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

ab178876 staining MEK1 + MEK2 in the human cell line HeLa (human cervix adenocarcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde, permabilised with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/100. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

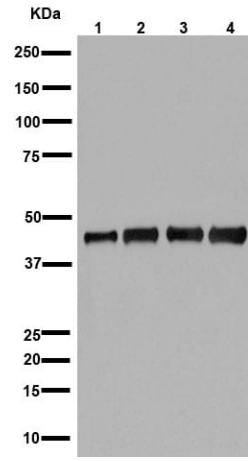
Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Immunocytochemistry/ Immunofluorescence - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embyro fibroblast cells) cells labeling MEK1 + MEK2 with ab178876 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/200 dilution (green). Cytoplasmic staining on NIH/3T3 cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows;
1. ab178876 at 1/100 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/200 dilution.



Western blot - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

All lanes : Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876) at 1/20000 dilution

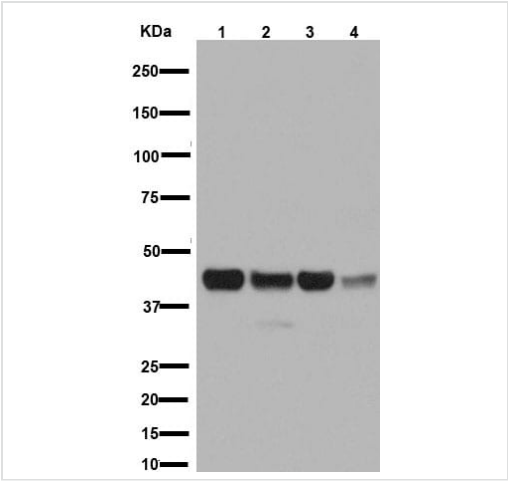
Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates
Lane 2 : 293T (Human epithelial cells from embryonic kidney) whole cell lysates
Lane 3 : A549 (Human lung carcinoma) whole cell lysates
Lane 4 : A431 (Human epidermoid carcinoma) whole cell lysates

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, 44 kDa
Observed band size: 44 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

All lanes : Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876) at 1/50000 dilution

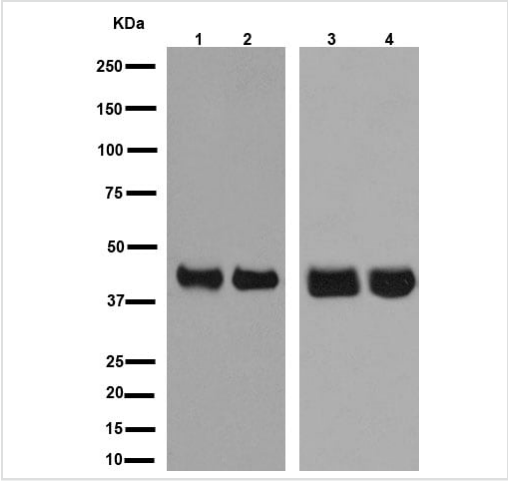
Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal heart lysate
Lane 3 : Human fetal kidney lysate
Lane 4 : Human fetal spleen lysate

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, 44 kDa
Observed band size: 44 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

All lanes : Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876) at 1/50000 dilution

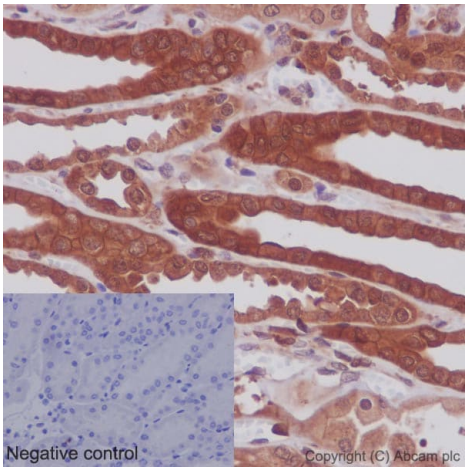
Lane 1 : Mouse brain lysate
Lane 2 : Rat brain lysate
Lane 3 : Mouse heart lysate
Lane 4 : Rat heart lysate

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, 44 kDa
Observed band size: 44 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

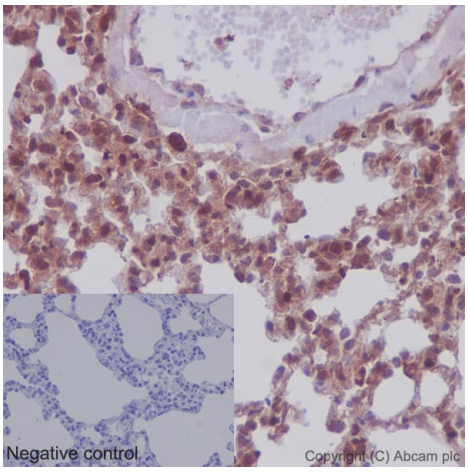


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

Immunohistochemical analysis of paraffin-embedded Human renal medulla tissue labeling MEK1 + MEK2 with ab178876 at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Renal tubule epithelial cells show strong cytoplasmic staining with some additional nuclear staining. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

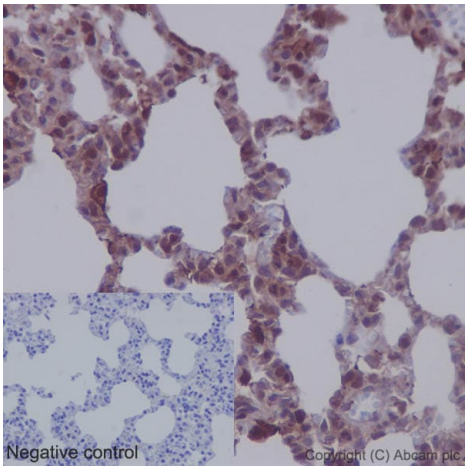


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labeling MEK1 + MEK2 with ab178876 at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Alveolar epithelial cells show strong cytoplasmic staining with some additional nuclear staining. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

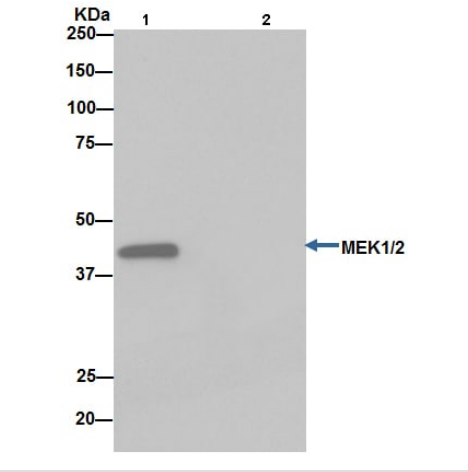


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

Immunohistochemical analysis of paraffin-embedded Rat lung tissue labeling MEK1 + MEK2 with ab178876 at 1/500 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Alveolar epithelial cells show strong cytoplasmic staining with some additional nuclear staining. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

MEK1 + MEK2 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell extract with ab178876 at 1/120 dilution. Western blot was performed from the immunoprecipitate using ab178876 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution. Lane 1: HeLa whole cell extract. Lane 2: PBS instead of HeLa whole cell extract.

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

Anti-MEK1 + MEK2 antibody [EPR16667] (ab178876)

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