abcam

ab32576

Anti-MEK1 antibody [Y77]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	Y77
Purification technique	Affinity purification Protein A
Specificity	The antibody does not crossreact with other MAP kinase kinase family members.
Concentration	0.328 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/20 - 1/50
Notes	-

WB

Tested

Species	Human
Dilution info	1/10000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/30 - 1/40
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

IHC-P

Tested

Species	Human
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information MAP2K1

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. Activates BRAF in a KSR1 or KSR2-dependent manner; by binding to KSR1 or KSR2 releases the inhibitory intramolecular interaction between KSR1 or KSR2 protein kinase and N-terminal domains which promotes KSR1 or KSR2-BRAF dimerization and BRAF activation (PubMed:29433126). 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.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	MEK1 also known as MAP2K1 is a dual-specificity kinase that plays a role in the MAPK/ERK pathway. It has a molecular weight of around 45 kDa. MEK1 phosphorylates and activates the extracellular signal-regulated kinases ERK1 and ERK2. MEK1 is expressed in various tissues but is especially abundant in brain and heart tissues. Researchers commonly measure MEK1 levels using techniques like MEK1 ELISA while phospho-MEK antibodies help analyze its activity state.
Biological function summary	MEK1 impacts cell processes such as proliferation differentiation and survival. It does not work alone but forms a complex with other proteins to exert its function. MEK1 activity is tightly controlled by upstream activators and downstream targets. One of the key phosphorylation sites on MEK1 is at serine 298 often marked as MEK1 pS298. This phosphorylation indicates MEK1's activated state which is critical for its biological function.
Pathways	MEK1 integrates signals within the MAPK/ERK pathway and interacts closely with RAF kinases upstream and ERK kinases downstream. This pathway modulates cellular responses to growth factors. MEK1 undergoes phosphorylation enhancing its activity and subsequently phosphorylates ERK proteins. Many studies isolate MEK1 activity using specific inhibitors such as the MEK1 inhibitor to dissect pathway dynamics. ERK inhibitor PD98059 also helps when studying parallel interactions with MEK1.
Associated diseases and disorders	MEK1 mutations or dysregulation have links to several types of cancer including melanoma. These often involve changes that lead to continuous MEK1 activation contributing to uncontrolled cell growth. MEK1 involvement in the RASopathies also shows its connection to disorders characterized by heart and facial abnormalities. In cancer MEK1 works closely with the BRAF protein which often harbors mutations leading to disease progression showcasing MEK1 as a target for therapeutic intervention.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

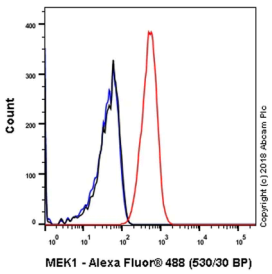
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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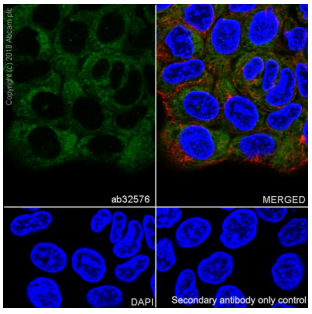
12 product images



Flow Cytometry (Intracellular) - Anti-MEK1 antibody [Y77] (ab32576)

MEK1 Flow Cytometry (Intracellular) staining using rabbit Anti-MEK1 antibody

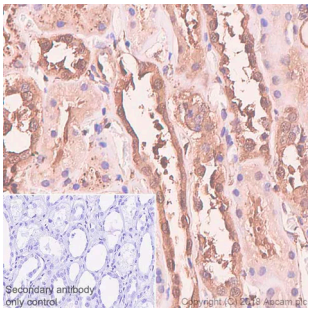
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling MEK1 with purified ab32576 at 1/30 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-MEK1 antibody [Y77] (ab32576)

MEK1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MEK1 antibody

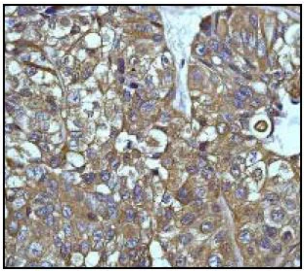
Immunocytochemistry/ Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling MEK1 with purified ab32576 at 1/50 dilution (6.6 µg/ml). Cells were fixed in 100% Methanol. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 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 (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK1 antibody [Y77] (ab32576)

MEK1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MEK1 antibody

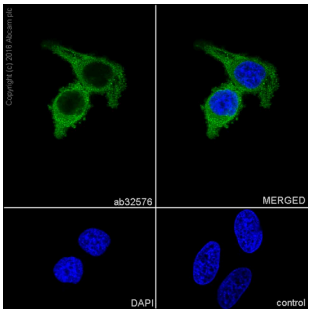
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human kidney tissue sections labeling MEK1 with purified ab32576 at 1/100 dilution (3.28 µg/ml). Heat mediated antigen retrieval was performed using heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK1 antibody [Y77] (ab32576)

MEK1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MEK1 antibody

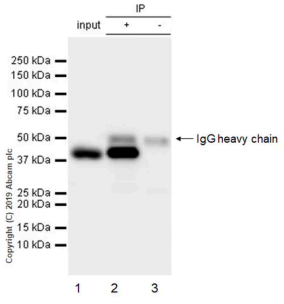
ab32576 (unpurified) at a 1/250 dilution staining MEK1 in human urinary bladder carcinoma tissue by IHC-P.



Immunocytochemistry/ Immunofluorescence - Anti-MEK1 antibody [Y77] (ab32576)

MEK1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MEK1 antibody

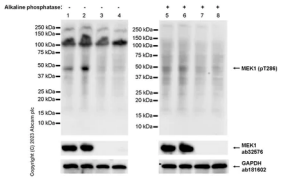
Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling MEK1 with purified ab32576 at 1/500 dilution (5 µg/ml). Cells were fixed with 4% PFA and permeabilized with 0.1% triton X-100. ab150077 Goat anti rabbit IgG (Alexa Fluor® 488) at 1/1000 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI. PBS was used instead of the primary antibody as the negative control.



Immunoprecipitation - Anti-MEK1 antibody [Y77] (ab32576)

ab32576 (purified) at 1/20 dilution (2ug) immunoprecipitating MEK1 in Jurkat whole cell lysate.
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10ug
Lane 2 (+): ab32576 & Jurkat whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32576 in Jurkat whole cell lysate
For western blotting, VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.
All lanes:
Immunoprecipitation - Anti-MEK1 antibody [Y77] (ab32576)

Predicted band size: 43 kDa



Western blot - Anti-MEK1 antibody [Y77] (ab32576)

Blocking and diluting buffer and concentration: 1% BSA/TBST. The identity of the higher MW bands between 100kDa and 200kDa are unknown. Lysates were freshly made and used for Western blotting immediately to minimize protein degradation. In Western blot, anti-GAPDH antibody (ab181602) loading control staining at 1/200000 dilution. In Western blot, anti-MEK1 antibody (ab32576) loading control staining at 1/1000 dilution. Exposure time: 15 seconds.
All lanes:
Western blot - Anti-MEK1 (phospho T286) antibody [EPR27226-8] (ab307509) at 1/1000 dilution

Lane 1:
Untreated wild-type HAP1(human chronic myelogenous leukemia near-haploid cell line) whole cell lysate (untreated membrane), at 20 µg

Lane 2:
Wild-type HAP1 treated with 100ng/ml nocodazole for 24h whole cell lysate (untreated membrane) at 20 µg

Lane 3:
untreated MEK1 knockout HAP1 whole cell lysate at 20 µg

Lane 4:
MEK1 knockout HAP1 treated with 100ng/ml nocodazole for 24h whole cell lysate at 20 µg

Lane 5:
Untreated wild-type HAP1 whole cell lysate (phosphatase treated membrane), at 20 µg

Lane 6:
Wild-type HAP1 treated with 100ng/ml nocodazole for 24h whole cell lysate (phosphatase treated membrane) at 20 µg

Lane 7:
Untreated MEK1 knockout HAP1 whole cell lysate (phosphatase treated membrane) at 20 µg

Lane 8:
MEK1 knockout HAP1 treated with 100ng/ml nocodazole for 24h whole cell lysate (phosphatase treated membrane) at 20 µg

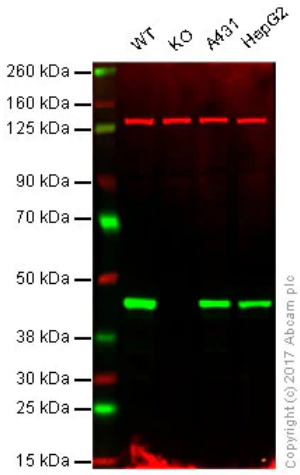
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 43 kDa

Observed band size: 43 kDa

Exposure time: 15s



Western blot - Anti-MEK1 antibody [Y77] (ab32576)

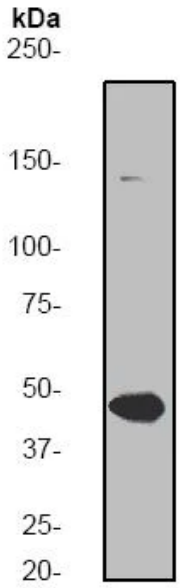
MEK1 Western blot staining using rabbit Anti-MEK1 antibody

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: MEK1 knockout HAP1 whole cell lysate (20 µg)
Lane 3: A431 whole cell lysate (20 µg)
Lane 4: HepG2 whole cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab32576 (unpurified) observed at 43 kDa. Red - loading control, ab18058, observed at 130 kDa.

ab32576 was shown to specifically react with MEK1 in wild-type HAP1 cells as signal was lost in MEK1 knockout cells. Wild-type and MEK1 knockout samples were subjected to SDS-PAGE. ab32576 and ab18058 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/10000 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.

All lanes:
Western blot - Anti-MEK1 antibody [Y77] (ab32576)

Predicted band size: 43 kDa



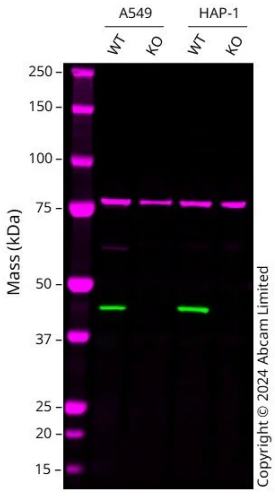
Western blot - Anti-MEK1 antibody [Y77] (ab32576)

All lanes:
Western blot - Anti-MEK1 antibody [Y77] (ab32576) at 1/10000 dilution

All lanes:
Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate

Predicted band size: 43 kDa

Observed band size: 45 kDa



Western blot - Anti-MEK1 antibody [Y77] (ab32576)

MEK1 Western blot staining using rabbit Anti-MEK1 antibody

Western blot: Anti-MAP2K1 antibody [Y77] (ab32576) staining at 1/10000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab32576 was shown to bind specifically to MAP2K1. A band was observed at 45 kDa in wild-type A549 cell lysates with no signal observed at this size in MAP2K1 knockout cell line. To generate this image, wild-type and MAP2K1 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

All lanes:
Western blot - Anti-MEK1 antibody [Y77] (ab32576) at 1/10000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
MAP2K1 knockout A549 cell lysate at 20 µg

Lane 3:
Wild-type HAP1 cell lysate at 20 µg

Lane 4:
MAP2K1 knockout HAP1 cell lysate at 20 µg

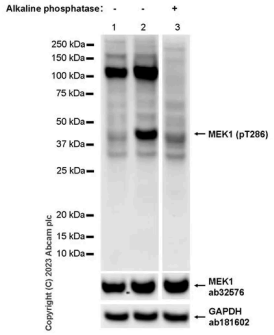
Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 43 kDa

Observed band size: 45 kDa



Western blot - Anti-MEK1 antibody [Y77] (ab32576)

Blocking and diluting buffer and concentration: 1% BSA/TBST. The identity of the higher MW bands between 100kDa and 200kDa are unknown. Lysates were freshly made and used for Western blotting immediately to minimize protein degradation. In Western blot, anti-GAPDH antibody (ab181602) loading control staining at 1/200000 dilution. In Western blot, anti-MEK1 antibody (ab32576) loading control staining at 1/1000 dilution. Exposure time: 26 seconds.
All lanes:
Western blot - Anti-MEK1 (phospho T286) antibody [EPR27226-8] (ab307509) at 1/1000 dilution

Lane 1:
Untreated HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
HeLa treated with 100ng/ml nocodazole for 24h whole cell lysate (untreated membrane) at 20 µg

Lane 3:
HeLa treated with 100ng/ml nocodazole for 24h whole cell lysate (phosphatase treated membrane at 20 µg

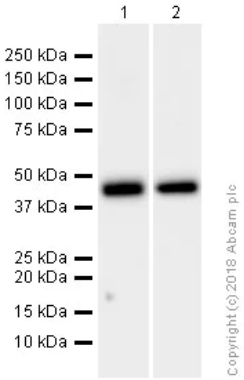
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 43 kDa

Observed band size: 43 kDa

Exposure time: 26s



Western blot - Anti-MEK1 antibody [Y77] (ab32576)

All lanes:
Western blot - Anti-MEK1 antibody [Y77] (ab32576) at 1/10000 dilution

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates at 20 µg

Lane 2:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 43 kDa

Observed band size: 45 kDa

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