abcam

Product datasheet

Anti-MEK1 antibody [Y77] ab32576

KO VALIDATED Recombinant RabMAb

17 References 10 Images

Overview

Product name	Anti-MEK1 antibody [Y77]
Description	Rabbit monoclonal [Y77] to MEK1
Host species	Rabbit
Specificity	The antibody does not crossreact with other MAP kinase kinase family members.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human MEK1 aa 350-450 (C terminal). The exact sequence is proprietary.
Positive control	WB: Wild-type HAP1 whole cell lysate; A431, Jurkat, HeLa and HepG2 whole cell lysates. IHC-P: Urinary bladder carcinoma tissue, human kidney tissue. ICC/IF: HeLa, A431 ells. IP: Jurkat cell lysate Flow Cyt (intra): HeLa 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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y77
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32576 in the following tested applications.

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

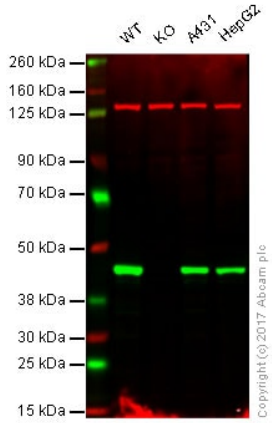
Application	Abreviews	Notes
Flow Cyt (Intra)		1/30 - 1/40. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50.
WB		1/10000. Detects a band of approximately 45 kDa (predicted molecular weight: 43 kDa).
IP		1/20 - 1/50.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

Target

Function	Catalyzes the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in MAP kinases. Activates ERK1 and ERK2 MAP kinases.
Tissue specificity	Widely expressed, with extremely low levels in brain.
Involvement in disease	Defects in MAP2K1 are a cause of cardiofaciocutaneous syndrome (CFC syndrome) [MIM:115150]; also known as cardio-facio-cutaneous syndrome. CFC syndrome is characterized by a distinctive facial appearance, heart defects and mental retardation. Heart defects include pulmonic stenosis, atrial septal defects and hypertrophic cardiomyopathy. Some affected individuals present with ectodermal abnormalities such as sparse, friable hair, hyperkeratotic skin lesions and a generalized ichthyosis-like condition. Typical facial features are similar to Noonan syndrome. They include high forehead with bitemporal constriction, hypoplastic supraorbital ridges, downslanting palpebral fissures, a depressed nasal bridge, and posteriorly angulated ears with prominent helices. The inheritance of CFC syndrome is autosomal dominant.
Sequence similarities	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase subfamily. Contains 1 protein kinase domain.
Post-translational modifications	Phosphorylation on Ser/Thr by MAP kinase kinase kinases (RAF or MEKK1) regulates positively the kinase activity. Acetylation by Yersinia yopJ prevents phosphorylation and activation, thus blocking the MAPK

signaling pathway.

Images

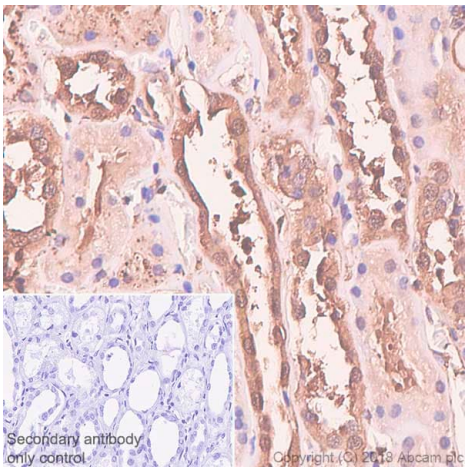


Western blot - Anti-MEK1 antibody [Y77] (ab32576)

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.



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

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.

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

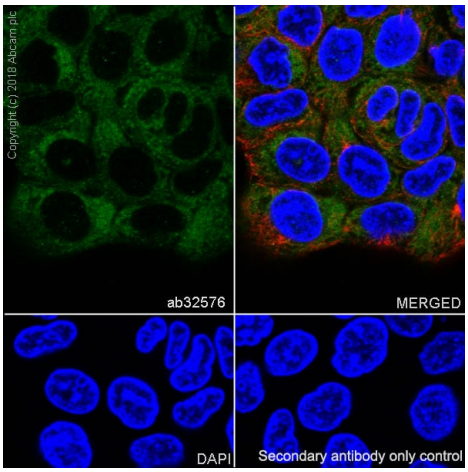
All lanes : Anti-MEK1 antibody [Y77] (ab32576) at 1/10000 dilution (Purified)

Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

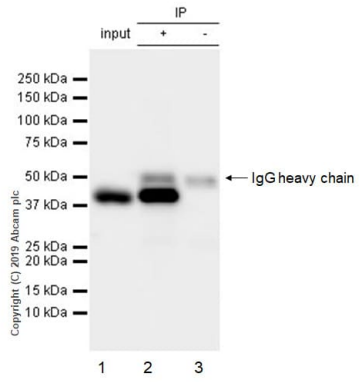
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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



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

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.



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

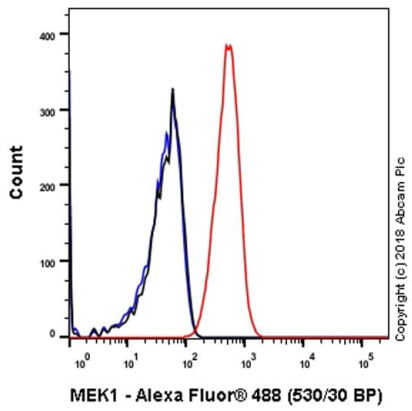
ab32576 (purified) at 1/20 dilution (2ug) immunoprecipitating MEK1 in Jurkat whole cell lysate. Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10ug

Lane 2 (+): ab32576 & Jurkat whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32576 in Jurkat whole cell lysate

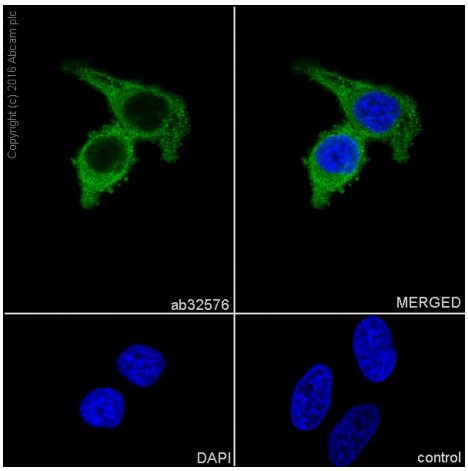
For western blotting, VeriBlot for IP secondary antibody (HRP) (**ab131366**) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



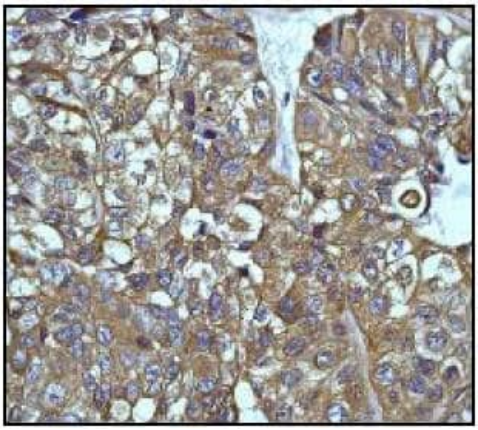
Flow Cytometry (Intracellular) - Anti-MEK1 antibody [Y77] (ab32576)

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 Fluor® 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).



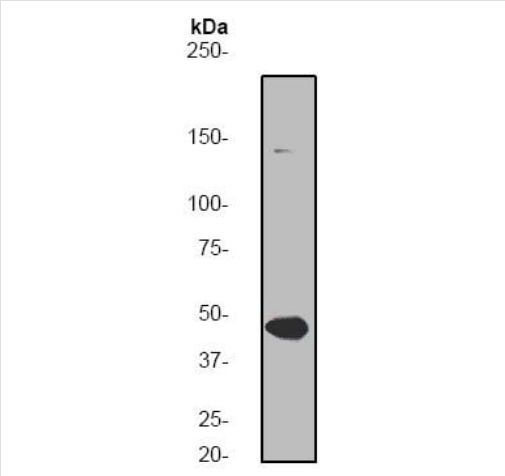
Immunocytochemistry/ Immunofluorescence - Anti-MEK1 antibody [Y77] (ab32576)

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.



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

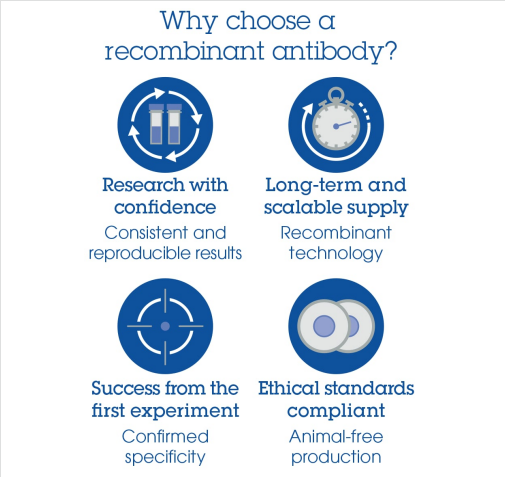
ab32576 (unpurified) at a 1/250 dilution staining MEK1 in human urinary bladder carcinoma tissue by IHC-P.



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

Anti-MEK1 antibody [Y77] (ab32576) at 1/10000 dilution (unpurified) + Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate

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



Anti-MEK1 antibody [Y77] (ab32576)

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