abcam

Product datasheet

Anti-MEK2 antibody [Y78] ab32517

KO VALIDATED Recombinant RabMAb

3 Abreviews 6 References 9 Images

Overview

Product name	Anti-MEK2 antibody [Y78]
Description	Rabbit monoclonal [Y78] to MEK2
Host species	Rabbit
Specificity	This antibody does not cross react with other MAP kinase kinase family members
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. within Human MEK2 aa 1-100 (N terminal). The exact sequence is proprietary. Database link: P36507
Positive control	WB: HEK-293T, HAP1, K562, Jurkat whole cell lysate (ab7899); Mouse brain and lung lysates. ICC/IF: HeLa and wildtype HAP1 cells. IHC-P: Human prostate carcinoma tissue.
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. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	Y78
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab32517 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)		1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use a concentration of 1 µg/ml.
WB	★★★★☆ (2)	1/10000. Detects a band of approximately 45 kDa (predicted molecular weight: 44 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Target

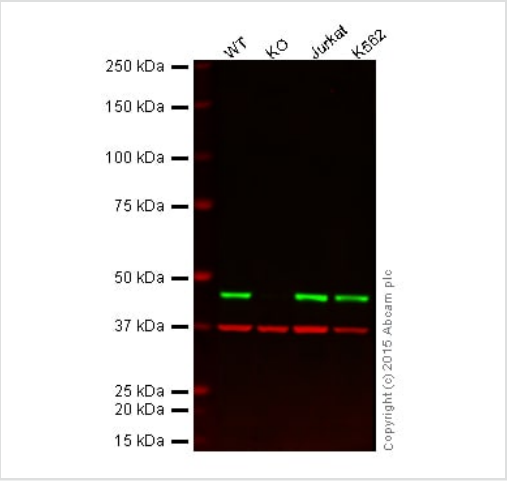
Function Catalyzes the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in MAP kinases. Activates the ERK1 and ERK2 MAP kinases.

Involvement in disease Defects in MAP2K2 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.

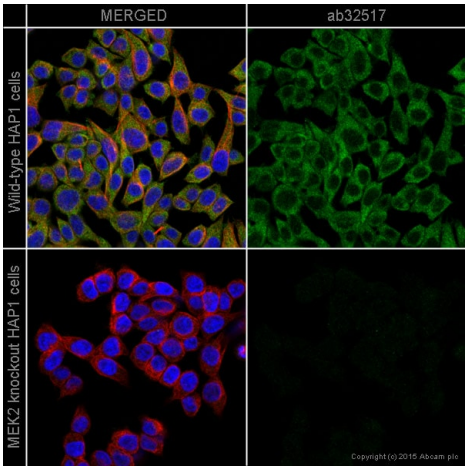
Post-translational modifications MAPKK is itself dependent on Ser/Thr phosphorylation for activity catalyzed by MAP kinase kinase kinases (RAF or MEKK1).
Acetylation of Ser-222 and Ser-226 by Yersinia yopJ prevents phosphorylation and activation, thus blocking the MAPK signaling pathway.

Images



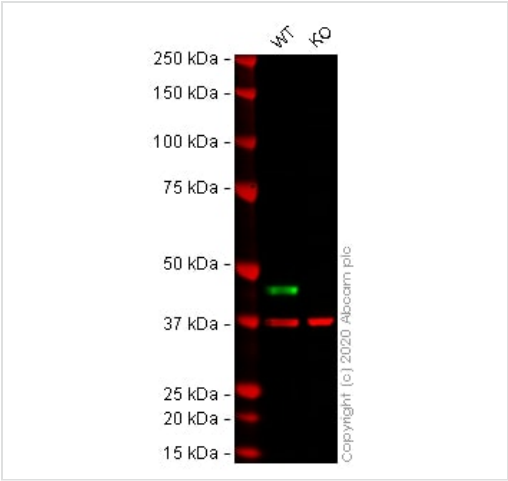
Western blot - Anti-MEK2 antibody [Y78] (ab32517)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: MEK2 knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate (20 µg)
Lane 4: K562 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab32517 observed at 44 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab32517 was shown to specifically react with MEK2 when MEK2 knockout samples were used. Wild-type and MEK2 knockout samples were subjected to SDS-PAGE. ab32517 and ab8245 (loading control to GAPDH) were diluted 1/10 000 and 1/2000 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 1/10 000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-MEK2 antibody [Y78] (ab32517)

ab32517 staining MEK2 in wild-type HAP1 cells (top panel) and MEK2 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab32517 at 1µg/ml and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Western blot - Anti-MEK2 antibody [Y78] (ab32517)

All lanes : Anti-MEK2 antibody [Y78] (ab32517) at 1/10000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : MAP2K2 knockout HEK293T cell lysate

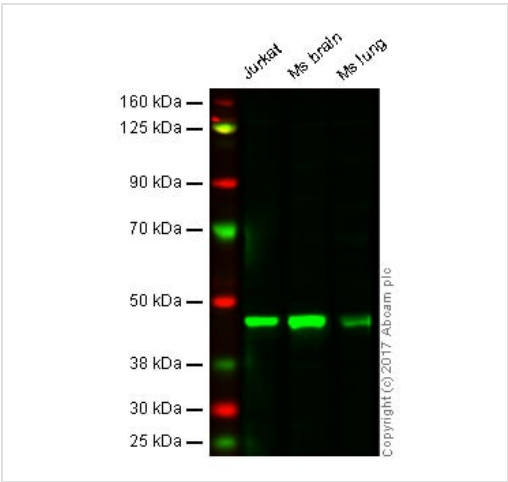
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1 - 2: Merged signal (red and green). Green - ab32517 observed at 45 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab32517 was shown to react with MEK2 in wild-type HEK-293T cells in western blot with loss of signal observed in MAP2K2 knockout cell line ab266315 (MAP2K2 knockout cell lysate ab257512). 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 ab32517 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 10000 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.



Western blot - Anti-MEK2 antibody [Y78] (ab32517)

All lanes : Anti-MEK2 antibody [Y78] (ab32517) at 1/10000 dilution

Lane 1 : Jurkat Whole Cell Lysate
Lane 2 : Mouse Brain Tissue Lysate
Lane 3 : Mouse Lung Tissue Lysate

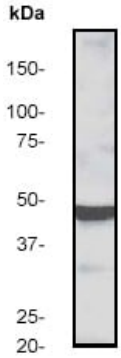
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Performed under reducing conditions.

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

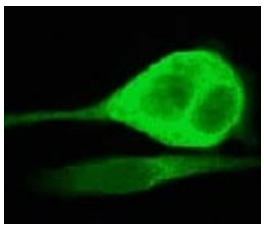
This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab32517 overnight at 4°C. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) at a 1:10000 dilution for 1hr at room temperature and then imaged.



Western blot - Anti-MEK2 antibody [Y78] (ab32517)

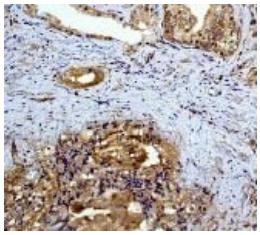
Anti-MEK2 antibody [Y78] (ab32517) at 1/10000 dilution + Jurkat cell lysate

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



Immunocytochemistry/ Immunofluorescence - Anti-MEK2 antibody [Y78] (ab32517)

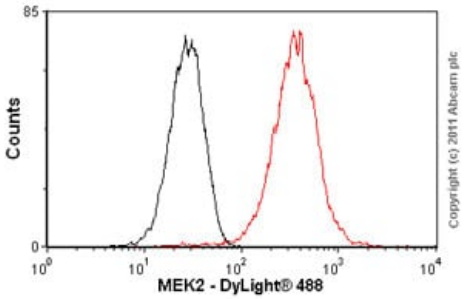
Immunofluorescent staining of HeLa cells using ab32517 at a dilution of 1/250.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK2 antibody [Y78] (ab32517)

Immunohistochemical analysis of paraffin embedded human prostate carcinoma using ab32517 at a dilution of 1/250.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-MEK2 antibody [Y78] (ab32517)

Overlay histogram showing HeLa cells stained with ab32517 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32517, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

Anti-MEK2 antibody [Y78] (ab32517)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery

- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors