abcam

Product datasheet

Anti-MEK2 antibody [Y78] - BSA and Azide free ab233731

KO VALIDATED Recombinant RabMAb

9 Images

Overview

Product name	Anti-MEK2 antibody [Y78] - BSA and Azide free
Description	Rabbit monoclonal [Y78] to MEK2 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody does not cross react with other MAP kinase kinase family members.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, K562, Jurkat and HEK-293T cell lysate; Mouse brain and lung lysates. ICC/IF: HeLa and wildtype HAP1 cells. IHC-P: Human prostate carcinoma tissue.
General notes	ab233731 is the carrier-free version of **ab32517**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y78
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab233731 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)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. 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.
ICC/IF		Use at an assay dependent concentration.

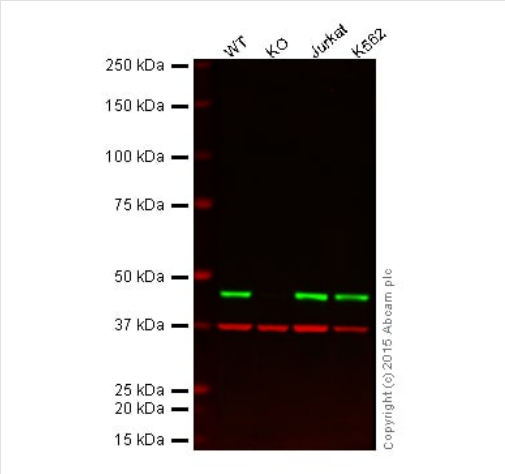
Target

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] - BSA and Azide free (ab233731)

This data was developed using **ab32517**, the same antibody clone in a different buffer formulation.

Lane 1 Wild-type HAP1 cell lysate (20 µg)

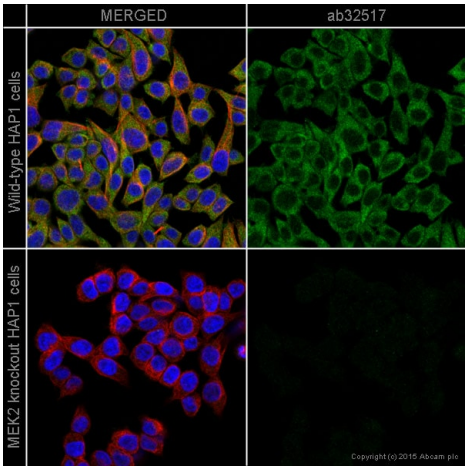
Lane 2 MEK2 knockout HAP1 cell lysate (20 µg)

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.

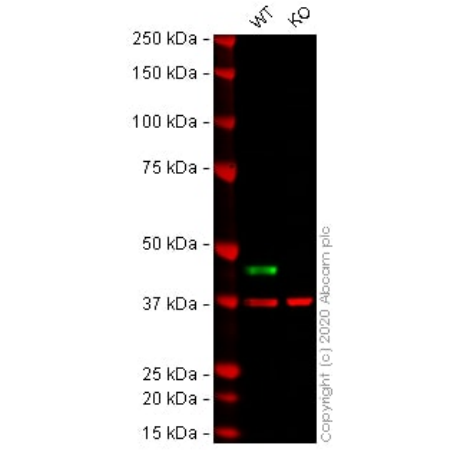
ab32517 was shown to specifically react with MEK2 when MEK2 knockout samples were used. Wild-type and MEK2 knockout samples were subjected to SDS-PAGE. **ab32517** and **ab8245** (loading control to GAPDH) were diluted 1/10 000 and 1/2000 and incubated overnight at 4°C. Blots were developed with Goat 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] - BSA and Azide free (ab233731)

This data was developed using **ab32517**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab233731)

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.

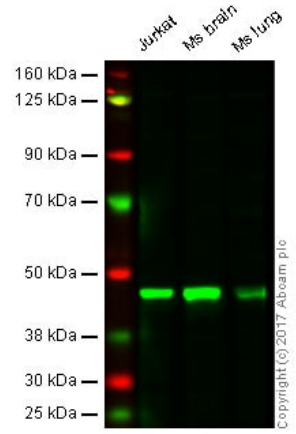
Predicted band size: 44 kDa
Observed band size: 45 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab32517**).

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] - BSA and Azide free (ab233731)

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.

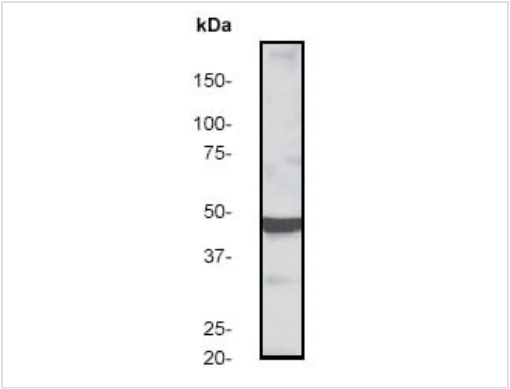
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Performed under reducing conditions.

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

This data was developed using **ab32517**, the same antibody clone in a different buffer formulation.

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.

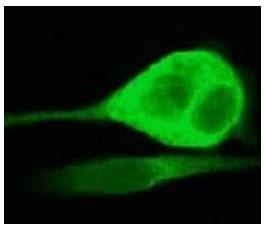


Western blot - Anti-MEK2 antibody [Y78] - BSA and Azide free (ab233731)

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

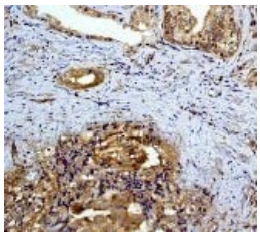
Predicted band size: 44 kDa
Observed band size: 45 kDa

This data was developed using **ab32517**, the same antibody clone in a different buffer formulation.



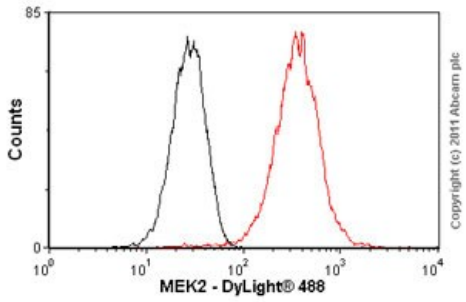
Immunocytochemistry/ Immunofluorescence - Anti-MEK2 antibody [Y78] - BSA and Azide free (ab233731)

This data was developed using **ab32517**, the same antibody clone in a different buffer formulation.Immunofluorescent staining of HeLa cells using **ab32517** at a dilution of 1/250.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK2 antibody [Y78] - BSA and Azide free (ab233731)

This data was developed using **ab32517**, the same antibody clone in a different buffer formulation.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.

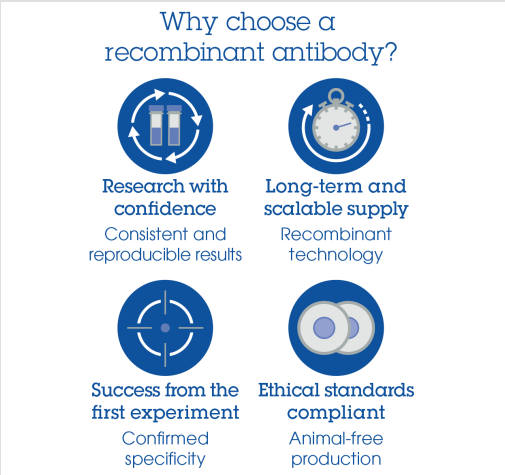


Flow Cytometry (Intracellular) - Anti-MEK2 antibody [Y78] - BSA and Azide free (ab233731)

This data was developed using **ab32517**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab233731)

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