abcam

Product datasheet

Anti-MEK1 antibody [E342] ab32091

KO VALIDATED Recombinant RabMAb

★★★★★ <u>5 Abreviews</u> <u>43 References</u> 6 Images

Overview

Properties

Product name	Anti-MEK1 antibody [E342]	
Description	Rabbit monoclonal [E342] to MEK1	
Host species	Rabbit	
Specificity	This antibody recognises MEK1, but 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: Human	
	Predicted to work with: Mouse, Rat, Cow, Dog 🛛 🔺	
Immunogen	Synthetic peptide within Human MEK1 aa 1-100 (N terminal). The exact sequence is proprietary. Database link: Q02750	
Positive control	WB: A431 cells and cell lysate. IHC-P: Human cervical carcinoma. ICC/IF: 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 <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>. 	

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	E342
lsotype	lgG

Applications

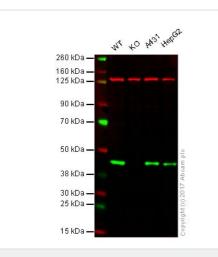
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab32091 in the following tested applications.

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

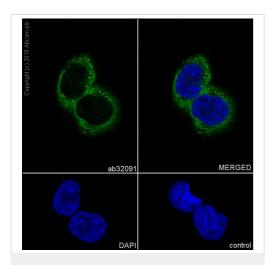
Application	Abreviews	Notes
Flow Cyt (Intra)		1/100. <u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★ ★ ★ ★ ★ (4)	1/1000 - 1/5000. Detects a band of approximately 45 kDa (predicted molecular weight: 43 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100 - 1/500.

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. 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.



Western blot - Anti-MEK1 antibody [E342] (ab32091)



Immunocytochemistry/ Immunofluorescence - Anti-MEK1 antibody [E342] (ab32091)

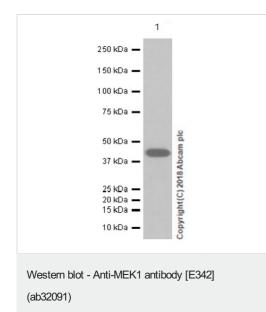
Lane 1: Wild-type HAP1 whole cell lysate (20 μg) Lane 2: MEK1 knockout HAP1 whole cell lysate (20 μg) Lane 3: A431 whole cell lysate (20 μg) Lane 4: HepG2 whole cell lysate (20 μg)

Lanes 1 - 4: Merged signal (red and green). Green - ab32091 observed at 43 kDa. Red - loading control, <u>ab18058</u>, observed at 130 kDa.

ab32091 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. Ab32091 and **ab18058** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 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.

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling MEK1 with purified ab32091 at dilution of 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, Alexa Fluor[®] 488-conjugated goat antirabbit IgG (1/1000) was used as the secondary antibody. Nuclei were counterstained with DAPI (blue).

Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



Anti-MEK1 antibody [E342] (ab32091) at 1/5000 dilution + A431 (Human epidermoid carcinoma epithelial cell)whole cell lysate at 20 µg

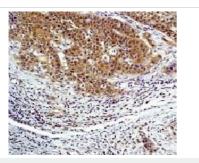
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

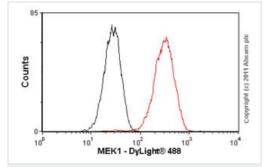
Predicted band size: 43 kDa Observed band size: 45 kDa

Exposure time: 15 seconds

Blocking/Diluting buffer and concentration: 5% NFDM /TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MEK1 antibody [E342] (ab32091) Ab32091, at a 1/100 dilution, staining MEK1 in paraffin embedded human cervical carcinoma tissue by Immunohistochemistry. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-MEK1 antibody [E342] (ab32091) Overlay histogram showing HeLa cells stained with ab32091 (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 (ab32091, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.



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