abcam

Product datasheet

Anti-MEK2 antibody [Y78] ab32517



★★★★★ 3 Abreviews 8 References 画像数 9

製品の概要

製品名 Anti-MEK2 antibody [Y78]

製品の詳細 Rabbit monoclonal [Y78] to MEK2

由来種 Rabbit

特異性 This antibody does not cross react with other MAP kinase kinase family members

アプリケーション 適用あり: Flow Cyt (Intra), ICC/IF, WB, IHC-P

種交差性 交差種: Mouse, Human

免疫原 Synthetic peptide. within Human MEK2 aa 1-100 (N terminal). The exact sequence is proprietary.

Database link: P36507

ポジティブ・コントロール 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.

特記事項 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.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 Y78 **アイソタイプ** lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab32517の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/100. ab172730 - Rabbit monoclonal lgG, 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.

ターゲット情報

配列類似性

翻訳後修飾

機能 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.

関連疾患 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.

Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase

Contains 1 protein kinase domain.

subfamily.

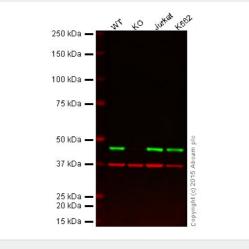
MAPKK is itself dependent on Ser/Thr phosphorylation for activity catalyzed by MAP kinase

kinase kinases (RAF or MEKK1).

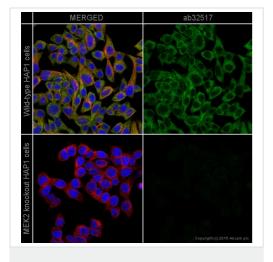
Acetylation of Ser-222 and Ser-226 by Yersinia yopJ prevents phosphorylation and activation,

thus blocking the MAPK signaling pathway.

画像



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



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

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

Lane 2: MEK2 knockout HAP1 cell lysate (20 µg)

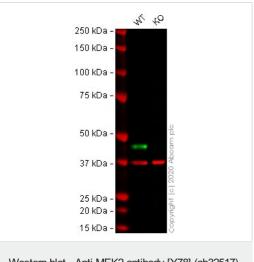
Lane 3: Jurkat cell lysate (20 µg)

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.

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

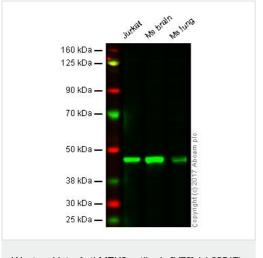
Lysates/proteins at 20 µg per lane.

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 lgG 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.

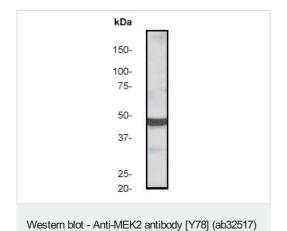
Secondary

All lanes: Goat anti-Rabbit lgG 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 lgG H&L (IRDye® 800CW) preabsorbed (ab216773) at a 1:10000 dilution for 1hr at room temperature and then imaged.



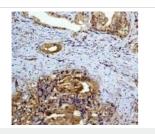
Anti-MEK2 antibody [Y78] (ab32517) at 1/10000 dilution + Jurkat cell lysate

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



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

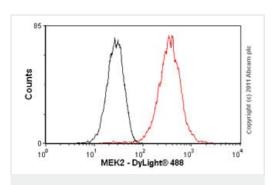
Immunocytochemistry/ Immunofluorescence - Anti-MEK2 antibody [Y78] (ab32517)



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

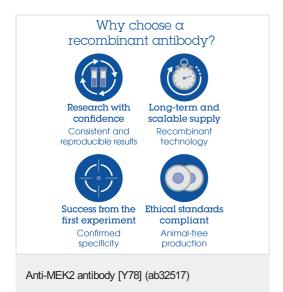
Immunohistochemical analysis of paraffin embedded human prostate carcinoma using ab32517 at a dilution of 1/250.

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 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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