abcam

Product datasheet

Anti-MAPK6/ERK3 antibody [EP1720Y] ab53277





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Overview

Product name Anti-MAPK6/ERK3 antibody [EP1720Y]

Description Rabbit monoclonal [EP1720Y] to MAPK6/ERK3

Host species Rabbit

Specificity The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

Tested applications Suitable for: WB, IHC-P

Unsuitable for: Flow Cyt or ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Synthetic peptide within Human MAPK6/ERK3 aa 1-100 (N terminal). The exact sequence is **Immunogen**

proprietary.

Database link: Q16659

Positive control WB: Wild-type HAP1, A431, C2C12, 2.4G2 and HeLa (ab150035) whole cell lysates, and rat

brain tissue lysates. IHC-P: Human kidney carcinoma and breast carcinoma tissues.

General notes The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

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**.

Properties

Form Liquid

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Storage instructions

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EP1720Y

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab53277 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
WB	★ ★ ★ ★ (1)	1/1000 - 1/10000. Detects a band of approximately 105 kDa (predicted molecular weight: 63 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. For unpurified use at 1/50

Application notes Is unsuitable for Flow Cyt or ICC/IF.

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Function Phosphorylates microtubule-associated protein 2 (MAP2). May promote entry in the cell cycle.

Tissue specificity Highest expression in the skeletal muscle, followed by the brain. Also found in heart, placenta,

lung, liver, pancreas, kidney and skin fibroblasts.

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase

subfamily.

Contains 1 protein kinase domain.

Domain The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the

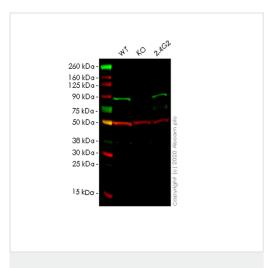
MAP kinases.

Post-translational

modifications

Dually phosphorylated on Thr-626 and Tyr-628, which activates the enzyme.

Images



Western blot - Anti-MAPK6/ERK3 antibody [EP1720Y] (ab53277) **All lanes :** Anti-MAPK6/ERK3 antibody [EP1720Y] (ab53277) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: MAPK6 knockout HeLa cell lysate

Lane 3: 2.4G2 (Rat B cell lymphoma B lymphocyte) cell lysate

Lysates/proteins at 20 µg per lane.

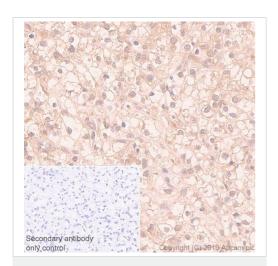
Secondary

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

Predicted band size: 63 kDa Observed band size: 90 kDa

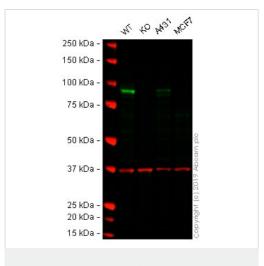
Lanes 1-3: Merged signal (red and green). Green - ab53277 observed at 90 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab53277 Anti-MAPK6/ERK3 antibody [EP1720Y] was shown to specifically react with MAPK6/ERK3 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab264910 (knockout cell lysate ab257526) was used. Wild-type and MAPK6/ERK3 knockout samples were subjected to SDS-PAGE. ab53277 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MAPK6/ERK3 antibody [EP1720Y] (ab53277)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney carcinoma tissue sections labeling MAPK6/ERK3 with Purified ab53277 at 1:100 dilution (13.52 µg/ml). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Purified)ImmunoHistoProbe one step HRP Polymer (ready to use) was used for detection. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-MAPK6/ERK3 antibody [EP1720Y] (ab53277)

All lanes : Anti-MAPK6/ERK3 antibody [EP1720Y] (ab53277) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: MAPK6 knockout HAP1 whole cell lysate

Lane 3: A431 whole cell lysate

Lane 4: MCF7 whole cell lysate

Lysates/proteins at 20 µg per lane.

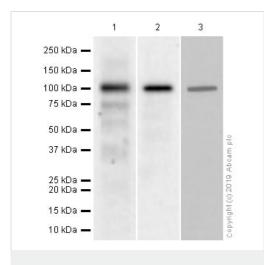
Performed under reducing conditions.

Predicted band size: 63 kDa **Observed band size:** 90 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab53277 observed at 90 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab53277 was shown to react with MAPK6 in HAP1 wild-type cells in Western blot. Loss of signal was observed when MAPK6 knockout sample was used. HAP1 wild-type and MAPK6 knockout whole cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween[®]) before incubation with

ab53277 and <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 800CW) preabsorbed (<u>ab216772</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-MAPK6/ERK3 antibody [EP1720Y] (ab53277) **All lanes :** Anti-MAPK6/ERK3 antibody [EP1720Y] (ab53277) at 1/1000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2: Rat brain lysates

Lane 3: C2C12 (Mouse myoblasts myoblast) whole cell lysates

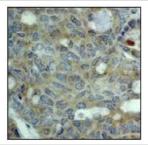
Lysates/proteins at 15 µg per lane.

Secondary

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

Predicted band size: 63 kDa **Observed band size:** 105 kDa

The molecular weight observed is consistent with what has been described in PMID:30642948 and 30166347



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MAPK6/ERK3 antibody [EP1720Y] (ab53277)

Unpurified ab53277 (1:50) staining human MAPK6/ERK3 in human breast carcinoma tissue by immunohistochemistry using paraffin embedded tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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