abcam

Product datasheet

Anti-MEKK2 antibody [EP626Y] ab33918

敲除验证 重组 RabMAb

★★★★★ 2 Abreviews 15 References 16 图像

概述

产品名称	Anti-MEKK2抗体[EP626Y]
描述	兔单克隆抗体[EP626Y] to MEKK2
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), IHC-Fr, ICC/IF, WB, IHC-P, IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. within Human MEKK2 aa 1-100 (N terminal). The exact sequence is proprietary. Database link: **Q9Y2U5**
阳性对照	WB: A549, HeLa, Hap1, C6, NIH/3T3, K562 and Jurkat (**ab7899**) whole cell lysates; Human breast carcinoma tissue lysate. Flow Cyt (intra): Jurkat and HepG2 cells. IHC-P: Human colon carcinoma, mouse and rat cerebral cortex. ICCIF: MCF-7 cell line. IP: HepG2 cell line.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	EP626Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab33918于以下的经测试应用**

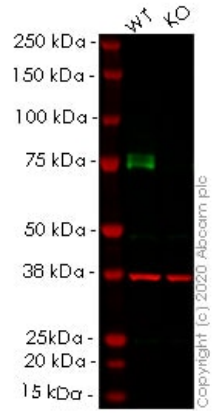
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/50 - 1/120. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-Fr		Use at an assay dependent concentration.
ICC/IF		1/250 - 1/500.
WB	★★★★★ (2)	1/10000 - 1/50000. Predicted molecular weight: 70 kDa.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/40.

靶标

功能	Component of a protein kinase signal transduction cascade. Regulates the JNK and ERK5 pathways by phosphorylating and activating MAP2K5 and MAP2K7 (By similarity). Plays a role in caveolae kiss-and-run dynamics.
序列相似性	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase kinase subfamily. Contains 1 OPR domain. Contains 1 protein kinase domain.
翻译后修饰	Autophosphorylated.
细胞定位	Cytoplasm. Nucleus. Upon EGF stimulation, translocates into the nucleus.

图片



Western blot - Anti-MEKK2 antibody [EP626Y] (ab33918)

All lanes : Anti-MEKK2 antibody [EP626Y] (ab33918) at 1/10000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : MAP3K2 knockout A549 cell lysate

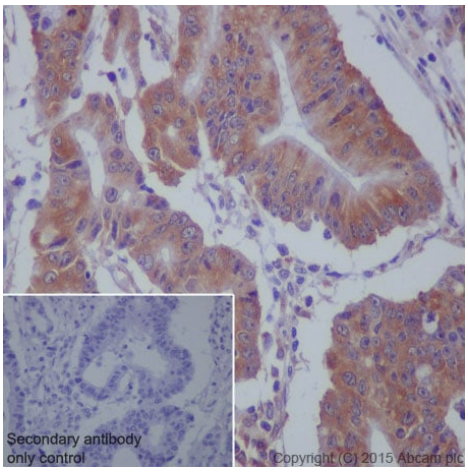
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 70 kDa
Observed band size: 75 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab33918 observed at 75 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

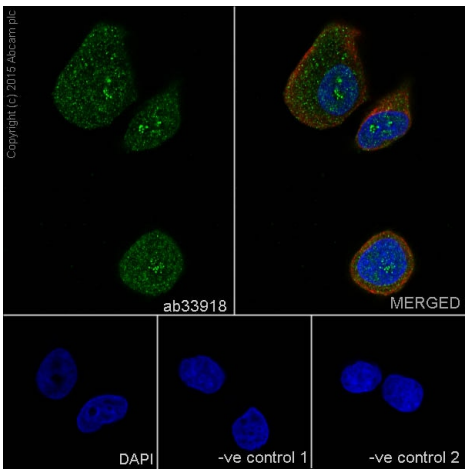
ab33918 was shown to react with MEKK2 in wild-type A549 cells in western blot. Loss of signal was observed when knockout cell line **ab267153** (knockout cell lysate **ab257522**) was used. Wild-type A549 and MAP3K2 knockout A549 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab33918 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 10000 dilution and a 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 paraffin-embedded sections) - Anti-MEKK2 antibody [EP626Y] (ab33918)

ab33918 staining MEKK2 in human colon carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.

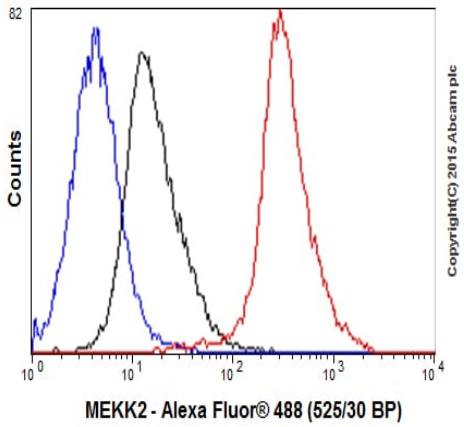
Negative control 1: PBS in place of primary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-MEKK2 antibody [EP626Y] (ab33918)

ab33918 staining MEKK2 in MCF-7 (human breast carcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) was used as the secondary antibody at a dilution of 1/1000. **ab7291** and **ab150120** were used as counterstains for primary antibody **ab75748** and secondary antibody **ab150077** respectively and DAPI was used as a nuclear counterstain.

Negative control 1: Rabbit primary antibody and anti-mouse secondary antibody (**ab150120**)
Negative control 2: Mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab150077**)

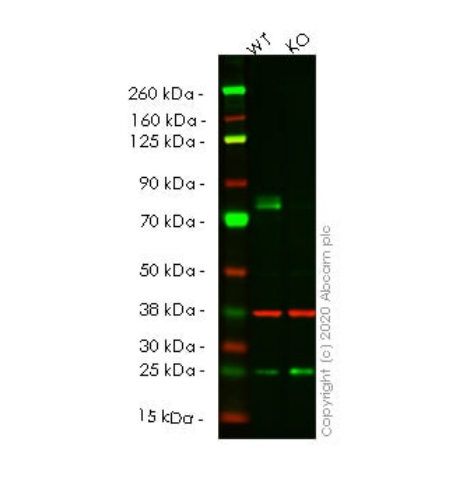


Flow Cytometry (Intracellular) - Anti-MEKK2 antibody [EP626Y] (ab33918)

ab33918 staining MEKK2 in Jurkat (human acute T cell leukemia) cellsby intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/120. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/500 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-MEKK2 antibody [EP626Y] (ab33918)

All lanes : Anti-MEKK2 antibody [EP626Y] (ab33918) at 1/10000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : MAP3K2 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

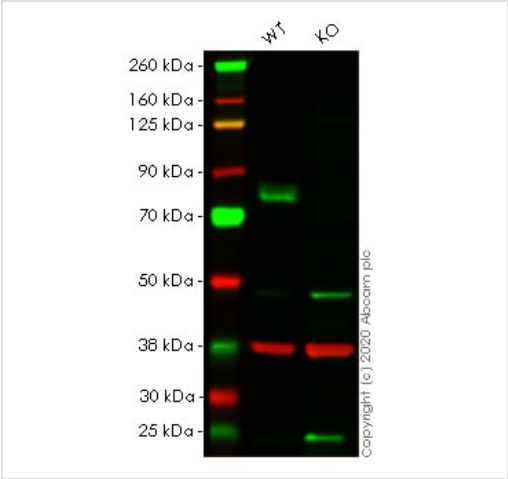
Performed under reducing conditions.

Predicted band size: 70 kDa
Observed band size: 75 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab33918 observed at 75 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab33918 was shown to react with MEKK2 in wild-type A549 cells in western blot. Loss of signal was observed when knockout cell line **ab267152** (knockout cell lysate **ab257521**) was used. Wild-type A549 and MAP3K2 knockout A549 cell lysates were subjected to

SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab33918 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 10000 dilution and a 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.



Western blot - Anti-MEKK2 antibody [EP626Y] (ab33918)

All lanes : Anti-MEKK2 antibody [EP626Y] (ab33918) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MAP3K2 knockout HeLa cell lysate

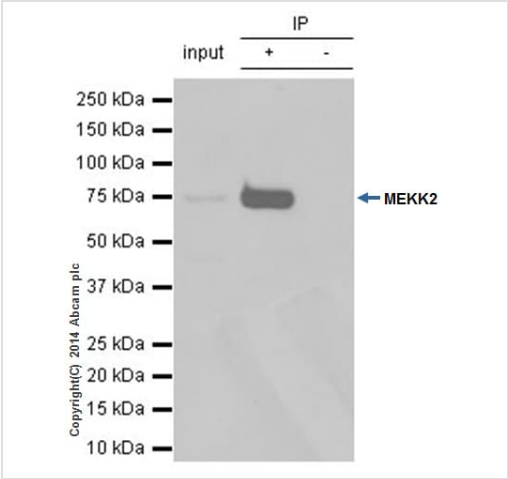
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 70 kDa
Observed band size: 75 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab33918 observed at 75 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

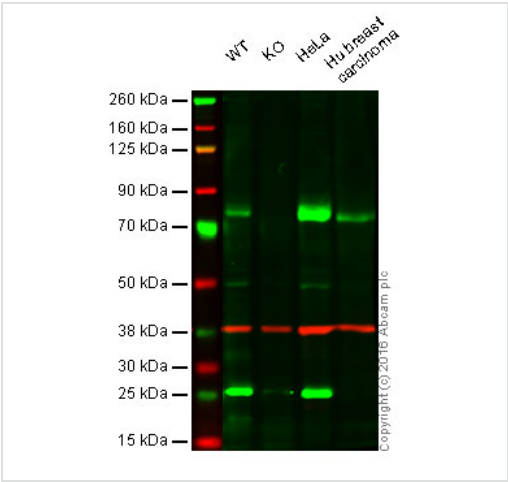
ab33918 was shown to react with MEKK2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264944** (knockout cell lysate **ab257520**) was used. Wild-type HeLa and MAP3K2 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab33918 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 10000 dilution and a 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.



Immunoprecipitation - Anti-MEKK2 antibody [EP626Y] (ab33918)

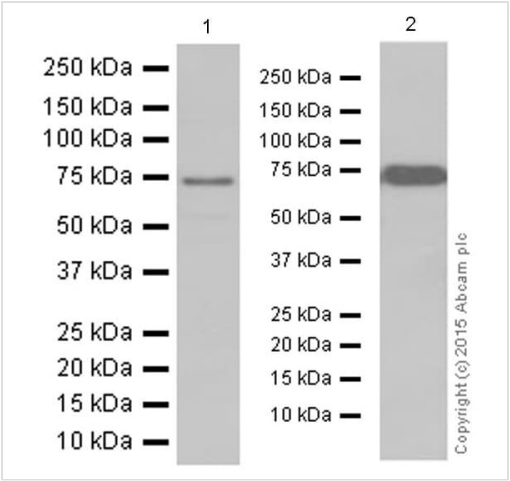
ab33918 immunoprecipitating MEKK2. 10µg of cell lysate was incubated with primary antibody at a dilution of 1/40 and VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at a dilution of 1/10000.

Lane 1: HepG2 (human hepatocellular carcinoma) whole cell lysate (10ug)
Lane 2: HepG2 (human hepatocellular carcinoma) whole cell lysate
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab33918 in HepG2 (human hepatocellular carcinoma) whole cell lysate



Western blot - Anti-MEKK2 antibody [EP626Y] (ab33918)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: MEKK2 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Human breast carcinoma lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab33918 observed at 75 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab33918 was shown to recognize MEKK2 when MEKK2 knockout samples were used, along with additional cross-reactive bands. Wild-type and MEKK2 knockout samples were subjected to SDS-PAGE. ab33918 and **ab8245** (loading control to GAPDH) were both diluted 1/10 000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) andGoat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-MEKK2 antibody [EP626Y] (ab33918)

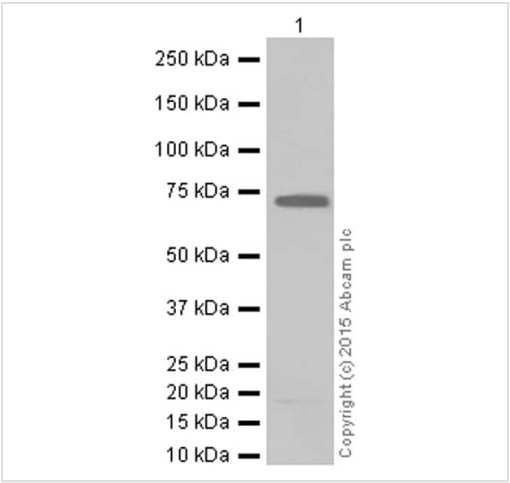
All lanes : Anti-MEKK2 antibody [EP626Y] (ab33918) at 1/20000 dilution

Lane 1 : C6 (rat glioma) whole cell lysate
Lane 2 : NIH/3T3 (mouse embryo) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 70 kDa

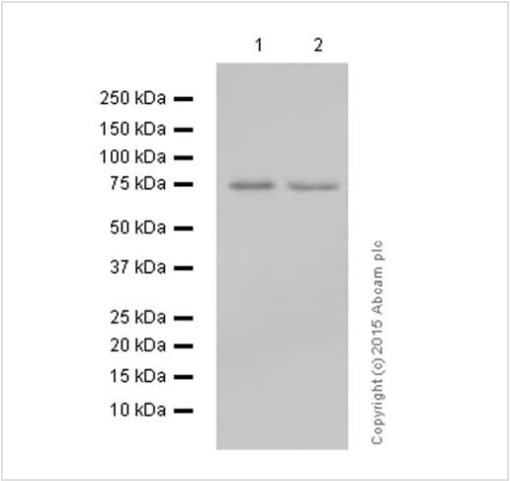


Western blot - Anti-MEKK2 antibody [EP626Y] (ab33918)

K562 (human chronic myelogenous leukemia) whole cell lysate at 10 µg

Secondary
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/20000 dilution

Predicted band size: 70 kDa



Western blot - Anti-MEKK2 antibody [EP626Y] (ab33918)

All lanes : Anti-MEKK2 antibody [EP626Y] (ab33918) at 1/20000 dilution

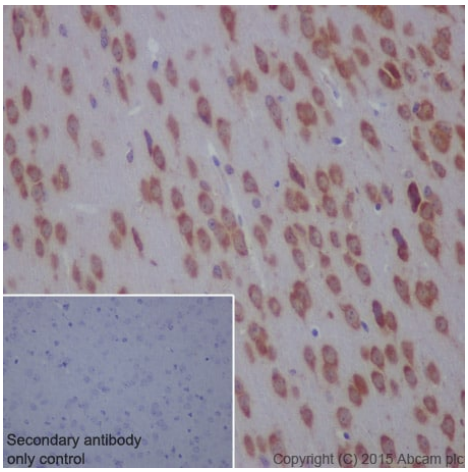
Lane 1 : Jurkat (human acute T cell leukemia) whole cell lysate
Lane 2 : HeLa (human cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Predicted band size: 70 kDa
Additional bands at: 70 kDa. We are unsure as to the identity of

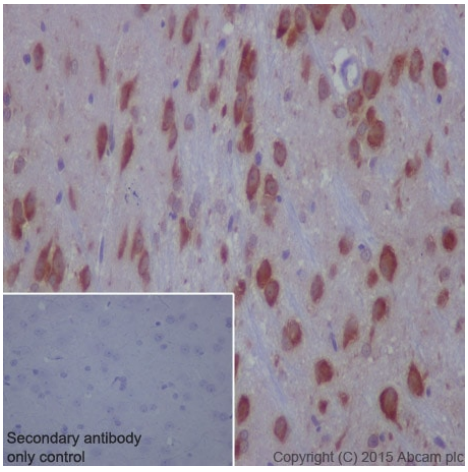
these extra bands.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEKK2 antibody [EP626Y] (ab33918)

ab33918 staining MEKK2 in mouse cerebral cortex tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.

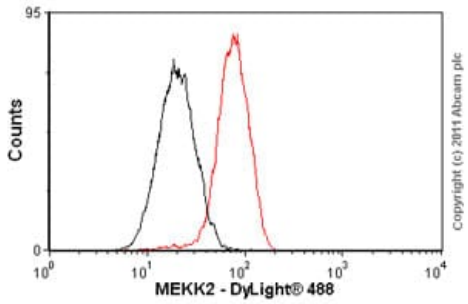
Negative control 1: PBS in place of primary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEKK2 antibody [EP626Y] (ab33918)

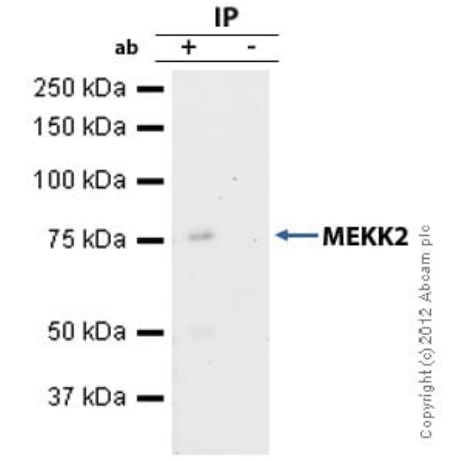
ab33918 staining MEKK2 in rat cerebral cortex tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.

Negative control 1: PBS in place of primary antibody.



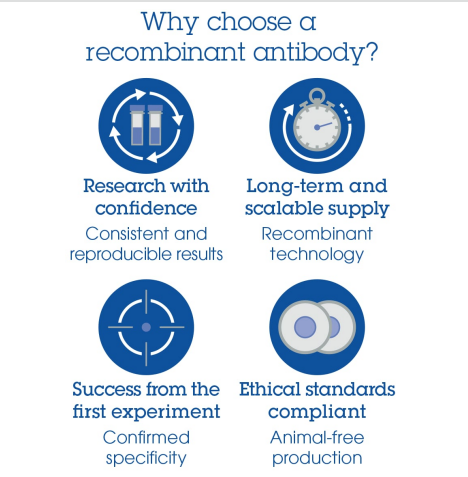
Flow Cytometry (Intracellular) - Anti-MEKK2 antibody [EP626Y] (ab33918)

Overlay histogram showing HepG2 cells stained with unpurified ab33918 (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 (ab33918, 1/50 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.



Immunoprecipitation - Anti-MEKK2 antibody [EP626Y] (ab33918)

MEKK2 was immunoprecipitated using 0.5mg HepG2 whole cell extract, 10µg of unpurified Rabbit monoclonal [EP626Y] to MEKK2 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, HepG2 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab33918.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**) .
Band: 75kDa: MEKK2.



Anti-MEKK2 antibody [EP626Y] (ab33918)

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