abcam

Product datasheet

Anti-p38 alpha/MAPK14 antibody [E229] ab170099

敲除验证 重组 RabMAb

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概述

产品名称	Anti-p38 alpha/MAPK14抗体[E229]
描述	兔单克隆抗体[E229] to p38 alpha/MAPK14
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, ICC/IF, IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human p38 aa 150-250 (internal sequence). The exact sequence is proprietary. Database link: **Q16539**
阳性对照	WB: Jurkat, C6, NIH/3T3 or HeLa whole cell lysate (**ab150035**). ICC/IF: NIH/3T3 cell lysate.
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 long term. Stable for 12 months at -20°C.
存储溶液	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	E229
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab170099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)		1/40.
WB	★★★★★ (3)	1/1000 - 1/5000. Predicted molecular weight: 42 kDa.
ICC/IF		1/100 - 1/250.
IP		1/10 - 1/100.

靶标

功能

Serine/threonine kinase which acts as an essential component of the MAP kinase signal transduction pathway. MAPK14 is one of the four p38 MAPKs which play an important role in the cascades of cellular responses evoked by extracellular stimuli such as proinflammatory cytokines or physical stress leading to direct activation of transcription factors. Accordingly, p38 MAPKs phosphorylate a broad range of proteins and it has been estimated that they may have approximately 200 to 300 substrates each. Some of the targets are downstream kinases which are activated through phosphorylation and further phosphorylate additionnal targets. RPS6KA5/MSK1 and RPS6KA4/MSK2 can directly phosphorylate and activate transcription factors such as CREB1, ATF1, the NF-kappa-B isoform RELA/NFKB3, STAT1 and STAT3, but can also phosphorylate histone H3 and the nucleosomal protein HMGN1. RPS6KA5/MSK1 and RPS6KA4/MSK2 play important roles in the rapid induction of immediate-early genes in response to stress or mitogenic stimuli, either by inducing chromatin remodeling or by recruiting the transcription machinery. On the other hand, two other kinase targets, MAPKAPK2/MK2 and MAPKAPK3/MK3, participate in the control of gene expression mostly at the post-transcriptional level, by phosphorylating ZFP36 (tristetraprolin) and ELAVL1, and by regulating EEF2K, which is important for the elongation of mRNA during translation. MKNK1/MNK1 and MKNK2/MNK2, two other kinases activated by p38 MAPKs, regulate protein synthesis by phosphorylating the initiation factor EIF4E2. MAPK14 interacts also with casein kinase II, leading to its activation through autophosphorylation and further phosphorylation of TP53/p53. In the cytoplasm, the p38 MAPK pathway is an important regulator of protein turnover. For example, CFLAR is an inhibitor of TNF-induced apoptosis whose proteasome-mediated degradation is regulated by p38 MAPK phosphorylation. In a similar way, MAPK14 phosphorylates the ubiquitin ligase SIAH2, regulating its activity towards EGLN3. MAPK14 may also inhibit the lysosomal degradation pathway of autophagy by interfering with the intracellular trafficking of the transmembrane protein ATG9. Another function of MAPK14 is to regulate the endocytosis of membrane receptors by different mechanisms that impinge on the small GTPase RAB5A. In addition, clathrin-mediated EGFR internalization induced by inflammatory cytokines and UV irradiation depends on MAPK14-mediated phosphorylation of EGFR itself as well as of RAB5A effectors. Ectodomain shedding of transmembrane proteins is regulated by p38 MAPKs as well. In response to inflammatory stimuli, p38 MAPKs phosphorylate the membrane-associated metalloprotease ADAM17. Such phosphorylation is required for ADAM17-mediated ectodomain shedding of TGF-alpha family ligands, which results in the activation of EGFR signaling and cell proliferation. Another p38

MAPK substrate is FGFR1. FGFR1 can be translocated from the extracellular space into the cytosol and nucleus of target cells, and regulates processes such as rRNA synthesis and cell growth. FGFR1 translocation requires p38 MAPK activation. In the nucleus, many transcription factors are phosphorylated and activated by p38 MAPKs in response to different stimuli. Classical examples include ATF1, ATF2, ATF6, ELK1, PTPRH, DDIT3, TP53/p53 and MEF2C and MEF2A. The p38 MAPKs are emerging as important modulators of gene expression by regulating chromatin modifiers and remodelers. The promoters of several genes involved in the inflammatory response, such as IL6, IL8 and IL12B, display a p38 MAPK-dependent enrichment of histone H3 phosphorylation on 'Ser-10' (H3S10ph) in LPS-stimulated myeloid cells. This phosphorylation enhances the accessibility of the cryptic NF-kappa-B-binding sites marking promoters for increased NF-kappa-B recruitment. Phosphorylates CDC25B and CDC25C which is required for binding to 14-3-3 proteins and leads to initiation of a G2 delay after ultraviolet radiation. Phosphorylates TIAR following DNA damage, releasing TIAR from GADD45A mRNA and preventing mRNA degradation. The p38 MAPKs may also have kinase-independent roles, which are thought to be due to the binding to targets in the absence of phosphorylation. Protein O-Glc-N-acylation catalyzed by the OGT is regulated by MAPK14, and, although OGT does not seem to be phosphorylated by MAPK14, their interaction increases upon MAPK14 activation induced by glucose deprivation. This interaction may regulate OGT activity by recruiting it to specific targets such as neurofilament H, stimulating its O-Glc-N-acylation. Required in mid-fetal development for the growth of embryo-derived blood vessels in the labyrinth layer of the placenta. Also plays an essential role in developmental and stress-induced erythropoiesis, through regulation of EPO gene expression. Isoform MXI2 activation is stimulated by mitogens and oxidative stress and only poorly phosphorylates ELK1 and ATF2. Isoform EXIP may play a role in the early onset of apoptosis.

组织特异性 Brain, heart, placenta, pancreas and skeletal muscle. Expressed to a lesser extent in lung, liver and kidney.

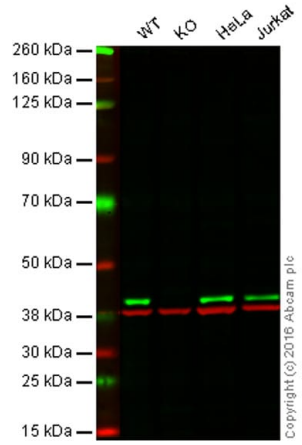
序列相似性 Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily.
Contains 1 protein kinase domain.

结构域 The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.

翻译后修饰 Dually phosphorylated on Thr-180 and Tyr-182 by the MAP2Ks MAP2K3/MKK3, MAP2K4/MKK4 and MAP2K6/MKK6 in response to inflammatory citokines, environmental stress or growth factors, which a ctivates the enzyme. Dual phosphorylation can also be mediated by TAB1-mediated autophosphorylation. TCR engagement in T-cells also leads to Tyr-323 phosphorylation by ZAP70. Dephosphorylated and inactivated by DUPS1, DUSP10 and DUSP16.
Acetylated at Lys-53 and Lys-152 by KAT2B and EP300. Acetylation at Lys-53 increases the affinity for ATP and enhances kinase activity. Lys-53 and Lys-152 are deacetylated by HDAC3.
Ubiquitinated. Ubiquitination leads to degradation by the proteasome pathway.

细胞定位 Cytoplasm. Nucleus.

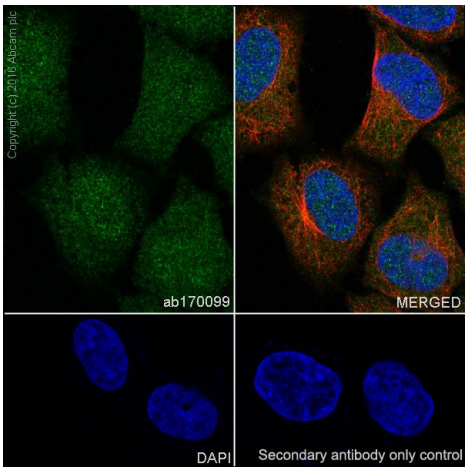
图片



Western blot - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: p38 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Jurkat cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab170099 observed at 40 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab170099 was shown to specifically react with p38 when p38 knockout samples were used. Wild-type and p38 knockout samples were subjected to SDS-PAGE. ab170099 and

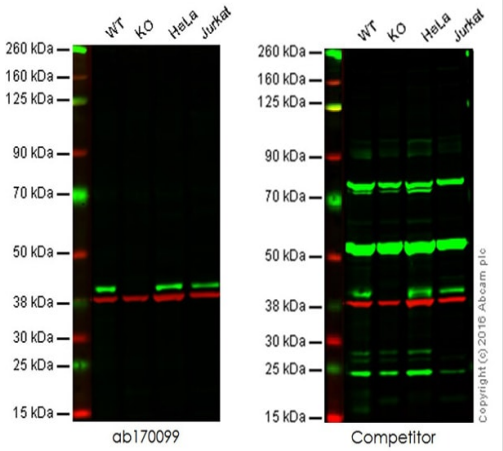
ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

Immunocytochemistry/ Immunofluorescence analysis of HeLa(Human epithelial cell line from cervix adenocarcinoma) cells labeling p38 with ab170099 at 1/250. Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% tritonX-100. **ab150077**, an AlexaFluor®488 Goat anti-Rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with **ab195889**, an anti-alpha tubulin antibody [DM1A] microtubule marker (Alexa Fluor® 594) at 1/200. Nuclei counterstained with DAPI (blue).

Confocal image shows nuclear and cytoplasmic staining on HeLa cell line.

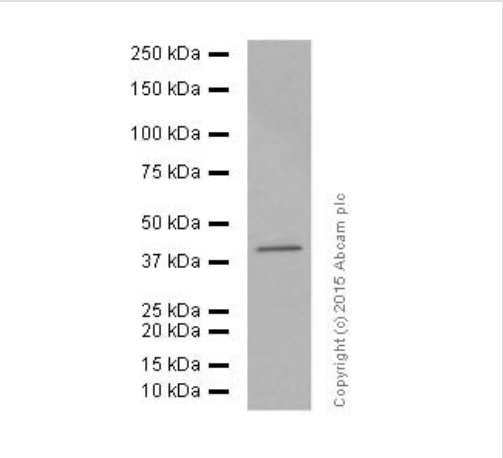


Western blot - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: p38 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Jurkat cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab170099 observed at 40 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab170099 and a competitor's top cited rabbit polyclonal antibody.



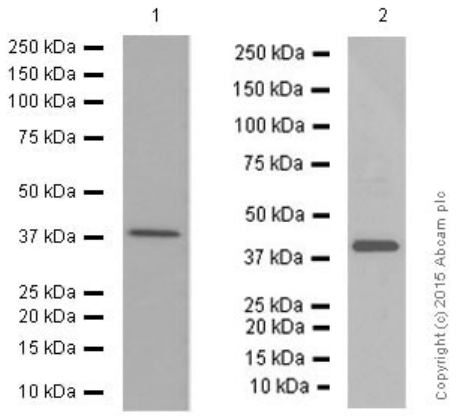
Western blot - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

Anti-p38 alpha/MAPK14 antibody [E229] (ab170099) at 1/5000 dilution (purified) + C6 cell lysate at 10 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 42 kDa

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



Western blot - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

All lanes : Anti-p38 alpha/MAPK14 antibody [E229] (ab170099) at 1/5000 dilution (purified)

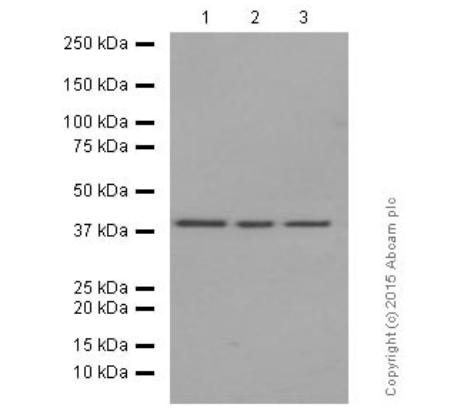
Lane 1 : Jurkat cell lysate
Lane 2 : HeLa cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 42 kDa

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



Western blot - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

All lanes : Anti-p38 alpha/MAPK14 antibody [E229] (ab170099) at 1/5000 dilution (purified)

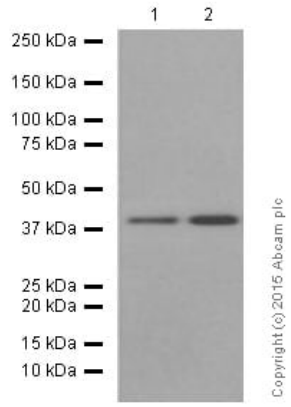
Lane 1 : NIH/3T3 cell lysate
Lane 2 : 3T3-L1 cell lysate
Lane 3 : PC-12 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
Lanes 1-2 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)
Lane 3 : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 20 µg

Predicted band size: 42 kDa

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



Western blot - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

All lanes : Anti-p38 alpha/MAPK14 antibody [E229] (ab170099) at 1/5000 dilution (purified)

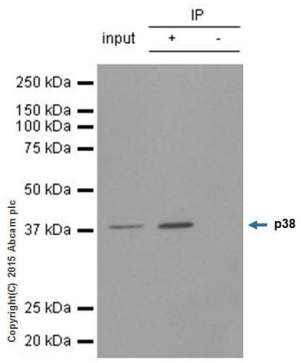
Lane 1 : MCF-7 cell lysate
Lane 2 : HEK293 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 42 kDa

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

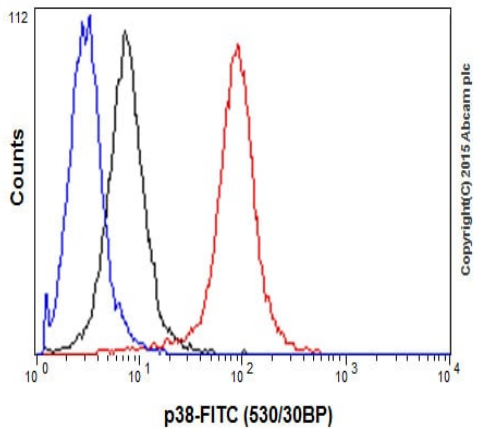


Immunoprecipitation - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

ab170099 (purified) at 1/20 immunoprecipitating p38 in Jurkat whole cell lysate. 10 ug of cell lysate was present in the input. For western blotting, a HRP-conjugated Veriblot for IP Detection Reagent (**ab131366**) (1/1,500) was used for detection. A rabbit monoclonal IgG (**ab172730**) was used intead of **ab128913** as a negative control (Lane 3).

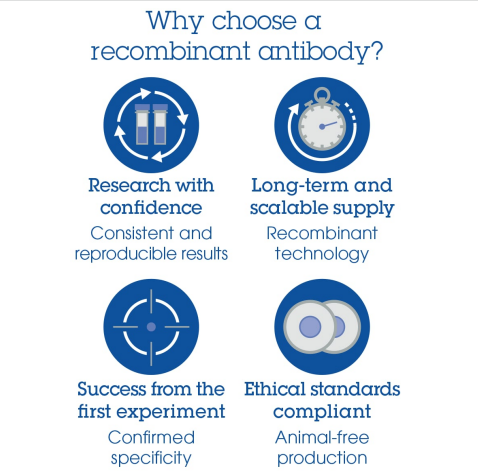
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

Intracellular Flow Cytometry analysis ofHeLa cells labelling p38 with purified ab170099 at 1/40 (red). Cells were fixed with 4% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Anti-p38 alpha/MAPK14 antibody [E229] (ab170099)

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