abcam

Product datasheet

Anti-p38 alpha/MAPK14 antibody [EPR16878] ab182453

KO 評価済 リコンビナント RabMAb

8 References 画像数 12

製品の概要

製品名	Anti-p38 alpha/MAPK14 antibody [EPR16878]
製品の詳細	Rabbit monoclonal [EPR16878] to p38 alpha/MAPK14
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, ICC/IF, IP
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HeLa, Jurkat, HEK-293T, Neuro-2a cell lysates; Human fetal heart, fetal kidney and fetal spleen lysates; Mouse heart, kidney and spleen lysates; Rat heart, kidney and spleen lysates; C6, RAW 264.7, PC12 and NIH 3T3 cell lysates. ICC/IF: HeLa and Jurkat cells. IP: Jurkat cells. Flow cyto(intra): Hap1 cells
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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% 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	EPR16878

アイソタイプ IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab182453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)		1/180.
WB		1/1000. Detects a band of approximately 38 kDa (predicted molecular weight: 41 kDa).
ICC/IF		1/500.
IP		1/100.

ターゲット情報

機能 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.

組織特異性

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

配列類似性

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

ドメイン

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

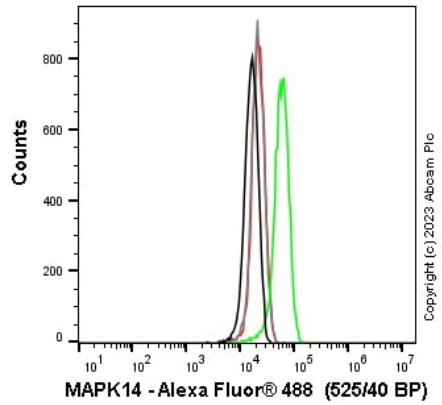
翻訳後修飾

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.

細胞内局在

Cytoplasm. Nucleus.

画像



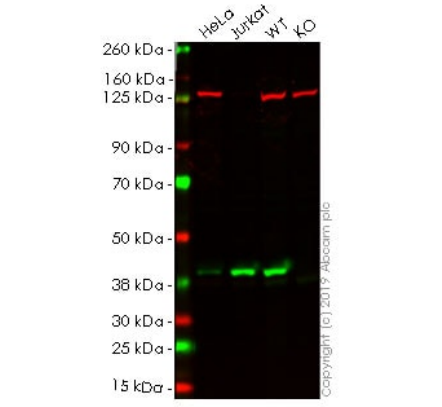
Flow Cytometry (Intracellular) - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

Flow cytometry overlay histogram showing wild-type Hap1 (green line) and MAPK14 knockout Hap1 stained with ab182453 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab182453) (1x 10^6 in 100µl at 0.2 µg/ml (1/11300)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, MAPK14 knockout Hap1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

All lanes : Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453) at 1/1000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : Wild-type HEK-293T cell lysate
Lane 4 : MAPK14 knockout HEK-293T cell lysate

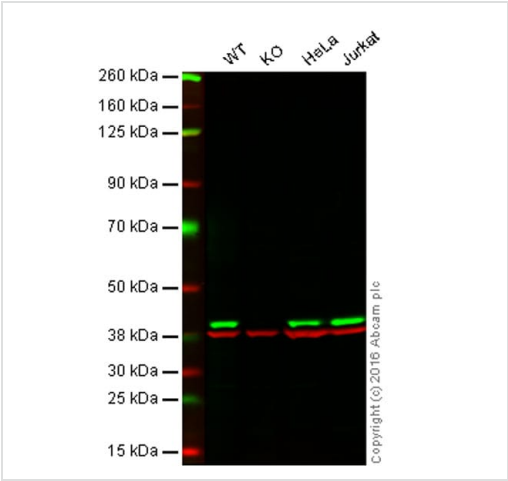
Lysates/proteins at 20 µg per lane.

Predicted band size: 41 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab182453 observed at 40 kDa. Red - loading control, **ab130007** observed at 125 kDa.

ab182453 was shown to react with p38 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab255406** (knockout cell lysate **ab263787**) was used. Wild-type and p38 knockout samples were subjected to SDS-PAGE. ab182453 and Anti-Vinculin antibody [VIN-54] (**ab130007**) were

incubated overnight at 4°C 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.



Western blot - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

All lanes : Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453) at 1/1000 dilution

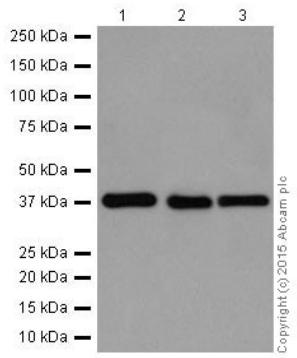
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : p38 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 41 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab182453 observed at 40 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab182453 was shown to specifically react with p38 when p38 knockout samples were used. Wild-type and p38 knockout samples were subjected to SDS-PAGE. ab182453 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.



Western blot - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

All lanes : Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 2 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate
Lane 3 : Neuro-2a (Mouse neuroblastoma cells) whole cell lysate

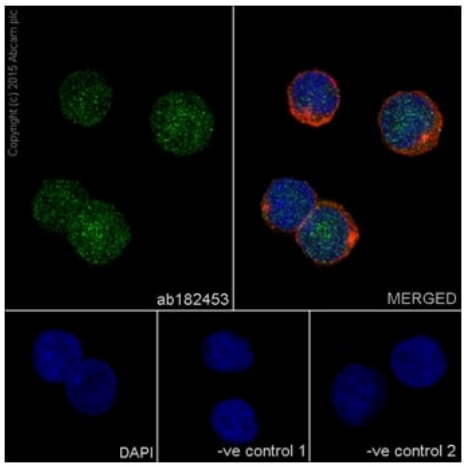
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

Predicted band size: 41 kDa
Observed band size: 38 kDa

Exposure time: 3 minutes

5% NFDM/TBST: Blocking and diluting buffer.

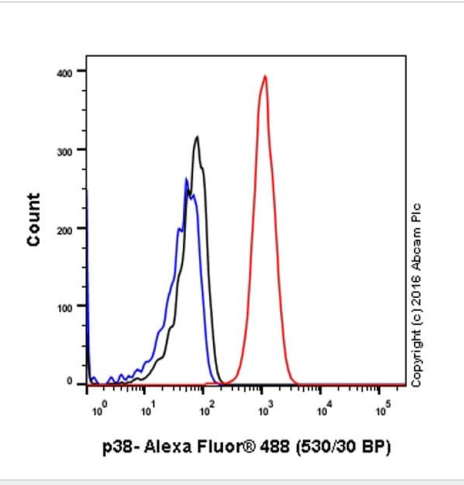


Immunocytochemistry/ Immunofluorescence - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling p38 with ab182453 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green).

Confocal image showing cytoplasm and nucleus staining on Jurkat cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:
1. ab182453 at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

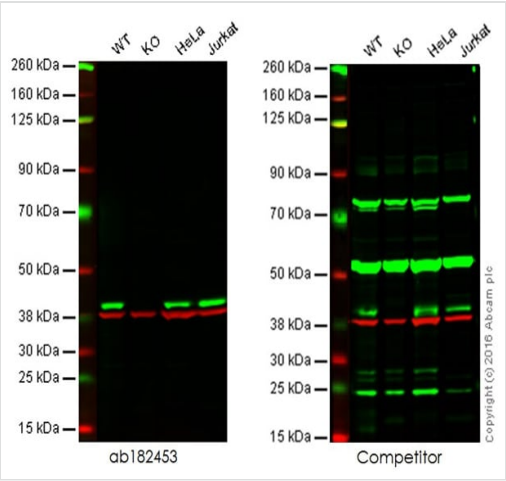


Flow Cytometry (Intracellular) - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

ab182453 staining p38in the human cell line Jurkat (human acute T cell leukemia) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/180. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 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-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

All lanes : Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

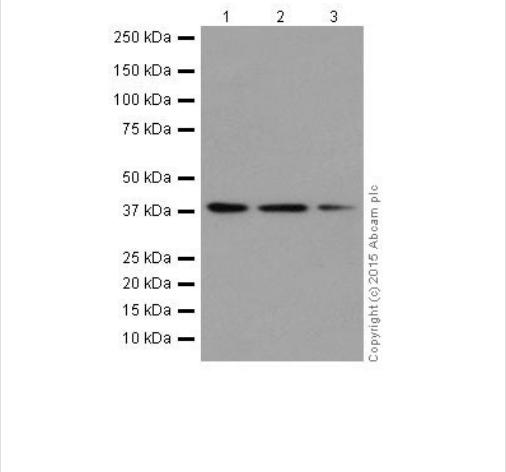
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : p38 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 41 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab182453 observed at 40 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab182453 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

All lanes : Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453) at 1/1000 dilution

Lane 1 : Human fetal heart lysate
Lane 2 : Human fetal kidney lysate
Lane 3 : Human fetal spleen lysate

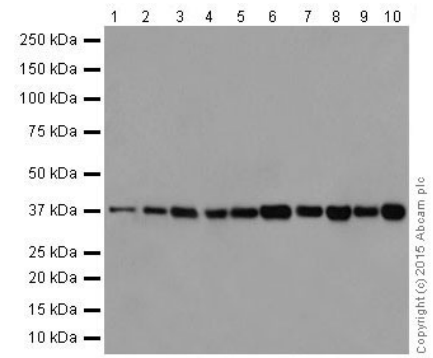
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 41 kDa
Observed band size: 38 kDa

Exposure time: 3 minutes

5% NFDM/TBST: Blocking and diluting buffer.



Western blot - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

All lanes : Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453) at 1/1000 dilution

Lane 1 : Mouse heart lysate
Lane 2 : Mouse kidney lysate
Lane 3 : Mouse spleen lysate
Lane 4 : Rat heart lysate
Lane 5 : Rat kidney lysate
Lane 6 : Rat spleen lysate
Lane 7 : C6 (Rat glial tumor cells) whole cell lysate
Lane 8 : RAW 264.7(Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate
Lane 9 : PC12 (Rat adrenal gland pheochromocytoma) whole cell lysate
Lane 10 : NIH 3T3 (Mouse embyro fibroblast cells) whole cell lysate

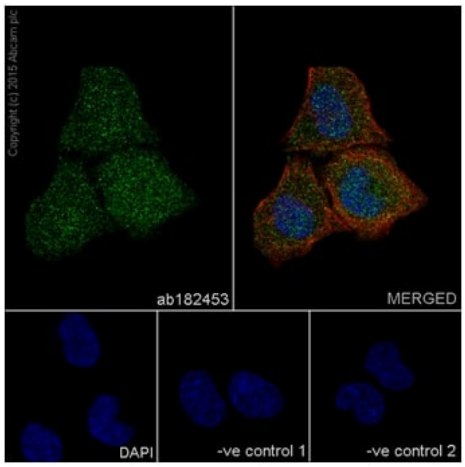
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/1000 dilution

Predicted band size: 41 kDa
Observed band size: 38 kDa

Exposure time: 30 seconds

5% NFDM/TBST: Blocking and diluting buffer.

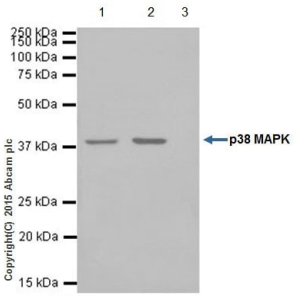


Immunocytochemistry/ Immunofluorescence - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling p38 with ab182453 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green).

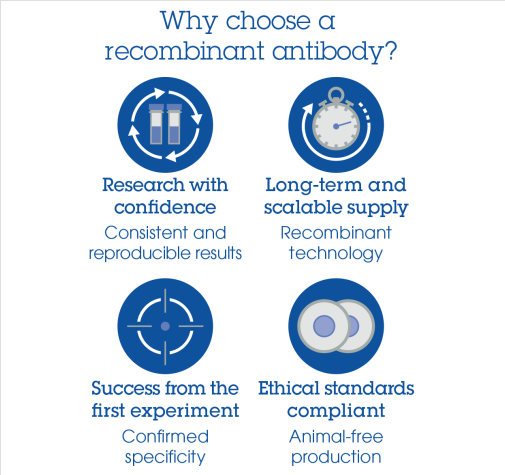
Confocal image showing cytoplasm and nucleus staining on HeLa cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:
1. ab182453 at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Immunoprecipitation - Anti-p38 alpha/MAPK14 antibody [EPR16878] (ab182453)

Immunoprecipitation of p38 from 1mg of Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate achieved using ab182453 at 1/100 dilution.
Lane 1: Input: 10µg of Jurkat whole cell lysate.
Lane 2: Jurkat whole cell lysate following IP with ab182453.
Lane 3: negative control: IP using Rabbit monoclonal IgG (**ab172730**) instead of ab182453 in Jurkat whole cell lysate.
Western blot was performed using ab182453 at 1/1000 dilution.
An Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1500 was used as secondary antibody.
Blocking and dilution buffer and concentration: 5% NFDM/TBST. 10 second exposure.



Anti-p38 alpha/MAPK14 antibody [EPR16878]

(ab182453)

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