

Product datasheet

p38 MAPK alpha (pT180/pY182) + total p38 MAPK alpha ELISA Kit ab126453

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Overview

Product name	p38 MAPK alpha (pT180/pY182) + total p38 MAPK alpha ELISA Kit
Detection method	Colorimetric
Sample type	Cell Lysate
Assay type	Semi-quantitative
Assay time	5h 00m
Assay duration	Multiple steps standard assay
Species reactivity	Reacts with: Mouse, Human
Product overview	<p>ab126453 is a very rapid, convenient and sensitive assay kit that can monitor the activation or function of important biological pathways in cell lysates. By determining phosphorylated p38 alpha protein in your experimental model system, you can verify pathway activation in your cell lysates. You can simultaneously measure numerous different cell lysates without spending excess time and effort in performing a Western Blot analysis.</p>

This Sandwich ELISA kit is an in vitro enzyme-linked immunosorbent assay for the measurement of human phospho-p38 alpha (Thr180/Tyr182) and total p38 alpha (help normalize the results of phospho-p38 alpha from different cell lysate being compared). An anti-p38 alpha (Thr180/Tyr182) (half plate, red marker on left side) and anti-total p38 alpha antibody (half plate, black marker on right side) has been coated onto a 96-well plate. Samples are pipetted into the wells and phosphorylated (left side) and total (right side) p38 alpha present in a sample is bound to the wells by the immobilized antibody. The wells are washed and rabbit anti p38 alpha is used to detect phosphorylated or total p38 alpha. After washing away unbound antibody, HRP-conjugated anti-rabbit IgG is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of p38 alpha (Thr180/Tyr182) or total p38 alpha bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Notes	<p>Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.</p> <p>It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.</p>
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Platform	Microplate
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Properties

Storage instructions

Store at -20°C. Please refer to protocols.

Components	1 x 96 tests
20X Wash Buffer	1 x 25ml
2X Cell Lysis Buffer	1 x 10ml
500X HRP-conjugated anti-rabbit IgG	1 x 25µl
5X Assay Diluent	1 x 15ml
Detection Antibody p38 alpha	2 vials
p38 alpha Microplate (12 strips x 8 wells)	1 unit
Positive Control: lyophilized powder from HeLa cell lysate	1 vial
Stop Solution	1 x 8ml
TMB One-Step Substrate Reagent	1 x 12ml

Function

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 additional 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- α 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- κ B-binding sites marking promoters for increased NF- κ 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.

Tissue specificity

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

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

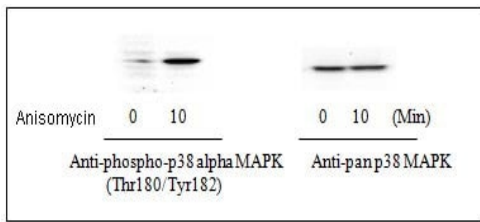
Dually phosphorylated on Thr-180 and Tyr-182 by the MAP2Ks MAP2K3/MKK3, MAP2K4/MKK4 and MAP2K6/MKK6 in response to inflammatory cytokines, environmental stress or growth factors, which activates the enzyme. Dual phosphorylation can also be mediated by TAB1-mediated autophosphorylation. TCR engagement 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.

Cellular localization

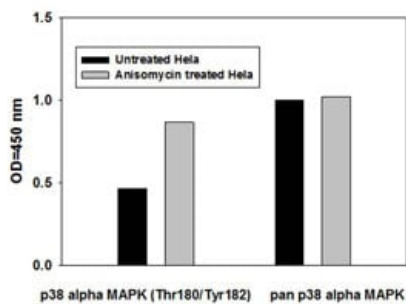
Cytoplasm. Nucleus.

Images



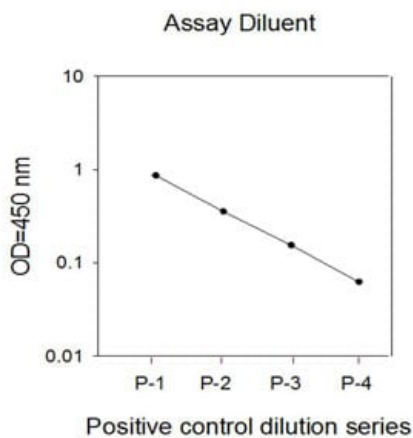
Western blot - p38 alpha (pT180/pY182) + total p38 alpha ELISA Kit (ab126453)

HeLa cells were treated or untreated with Anisomycin for 10 min at 37°C. Cell lysates were analyzed by Western Blot.



Sandwich ELISA - p38 alpha (pT180/pY182) + total p38 alpha ELISA Kit (ab126453)

HeLa cells were treated or untreated with Anisomycin for 10 min at 37°C. Cell lysates were analyzed using this phosphoELISA.



Sandwich ELISA - p38 alpha (pT180/pY182) + total p38 alpha ELISA Kit (ab126453)

HeLa cells were treated with Anisomycin at 37°C for 10 min. Solubilize cells at 4×10^7 cells/ml in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA.

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