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phospho-MLK3 (Thr277 + Ser281) Rabbit pAb

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Catalog # AP94722

Product Information

Application	IHC-P, IHC-F, IF
Primary Accession	Q16584
Reactivity	Human, Mouse, Rat
Predicted	Dog, Pig, Rabbit, Guinea Pig
Host	Rabbit
Clonality	Polyclonal
Calculated MW	92688
Physical State	Liquid
Immunogen	KLH conjugated synthesised phosphopeptide derived from human MLK3 around the phosphorylation site of Thr277/Ser281
Epitope Specificity	K(p-T)TQM(p-S)AA
Isotype	IgG
Purity	affinity purified by Protein A
Buffer	0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol.
SUBCELLULAR LOCATION	Cytoplasm, cytoskeleton, centrosome. Note=Location is cell cycle dependent.
SIMILARITY	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase kinase subfamily. Contains 1 protein kinase domain. Contains 1 SH3 domain.
SUBUNIT	Homodimer; undergoes dimerization during activation.
Post-translational modifications	Autophosphorylation on serine and threonine residues within the activation loop plays a role in enzyme activation. Thr-277 is likely to be the main autophosphorylation site. Phosphorylation of Ser-555 and Ser-556 is induced by CDC42.
Important Note	This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.
Background Descriptions	Members of the mixed-lineage kinase (MLK) family (including MLK1, MLK2, MLK3, and dual leucine zipper kinase [DLK]) are serine/threonine protein kinases that are expressed in multiple cell types. MLK3 is activated by phosphorylation in response to stress stimuli (e.g., inflammatory responses, UV, chemical stress) that are coupled to the small GTPase, Cdc42/rac. MLK3 is a multifunctional kinase that plays an essential role in several signaling pathways, including mitogen-activated protein kinase (i.e. activation of JNK and p38), IkappaB/NFkappaB, and p70 S6 kinase. Indeed MLK3 signaling occurs through multiple signaling domains in this protein kinase including (from N- to C-terminal) a glycine-rich domain, Src homology 3 (SH3) domain, a kinase domain, a zipper domain, a Cdc42/rac interactive binding (CRIB) domain and a Pro/Ser/Thr-rich domain. Phosphorylation of MLK3 occurs on multiple residues including threonine 277 and serine 281 within the activation loop of the kinase domain.

Additional Information

Gene ID	4296
Other Names	Mitogen-activated protein kinase kinase kinase 11, 2.7.11.25, Mixed lineage kinase 3, Src-homology 3 domain-containing proline-rich kinase, MAP3K11 (HGNC:6850)
Target/Specificity	Expressed in a wide variety of normal and neoplastic tissues including fetal lung, liver, heart and kidney, and adult lung, liver, heart, kidney, placenta, skeletal muscle, pancreas and brain.
Dilution	IHC-P=1:100-500,IHC-F=1:100-500,IF=1:100-500,Flow-Cyt=1ug/Test
Storage	Store at -20 °C for one year. Avoid repeated freeze/thaw cycles. When reconstituted in sterile pH 7.4 0.01M PBS or diluent of antibody the antibody is stable for at least two weeks at 2-4 °C.

Protein Information

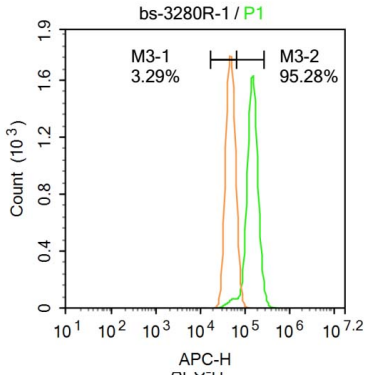
Name	MAP3K11 (HGNC:6850)
Function	Activates the JUN N-terminal pathway. Required for serum- stimulated cell proliferation and for mitogen and cytokine activation of MAPK14 (p38), MAPK3 (ERK) and MAPK8 (JNK1) through phosphorylation and activation of MAP2K4/MKK4 and MAP2K7/MKK7. Plays a role in mitogen- stimulated phosphorylation and activation of BRAF, but does not phosphorylate BRAF directly. Influences microtubule organization during the cell cycle.
Cellular Location	Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Note=Location is cell cycle dependent
Tissue Location	Expressed in a wide variety of normal and neoplastic tissues including fetal lung, liver, heart and kidney, and adult lung, liver, heart, kidney, placenta, skeletal muscle, pancreas and brain.

Background

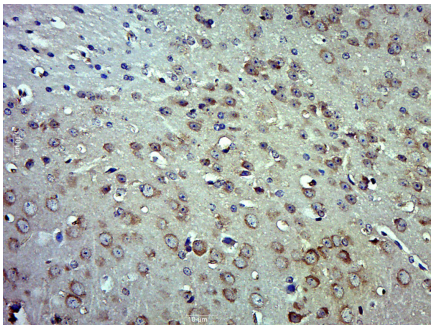
Members of the mixed-lineage kinase (MLK) family (including MLK1, MLK2, MLK3, and dual leucine zipper kinase [DLK]) are serine/threonine protein kinases that are expressed in multiple cell types. MLK3 is activated by phosphorylation in response to stress stimuli (e.g., inflammatory responses, UV, chemical stress) that are coupled to the small GTPase, Cdc42/rac. MLK3 is a multifunctional kinase that plays an essential role in several signaling pathways, including mitogen-activated protein kinase (i.e. activation of JNK and p38), IkappaB/NFkappaB, and p70 S6 kinase. Indeed MLK3 signaling occurs through multiple signaling domains in this protein kinase including (from N- to C-terminal) a glycine-rich domain, Src homology 3 (SH3) domain, a kinase domain, a zipper domain, a Cdc42/rac interactive binding (CRIB) domain and a Pro/Ser/Thr-rich domain. Phosphorylation of MLK3 occurs on multiple residues including threonine 277 and serine 281 within the activation loop of the kinase domain.

Images

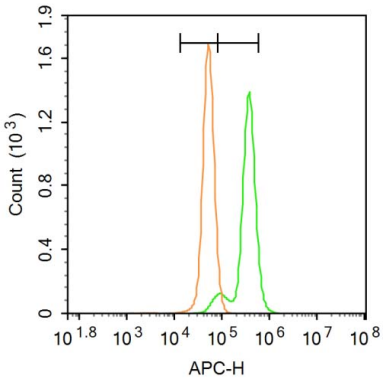
Blank control (Black line): A431 (Black).
Primary Antibody (green line): Rabbit Anti-MLK3 antibody (AP94722)
Dilution: 1 µg /10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG .
Secondary Antibody (white blue line): Goat anti-rabbit



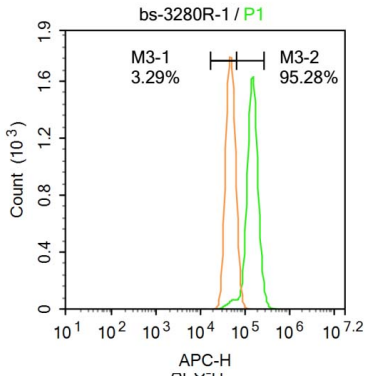
IgG-AF647
Dilution: 1 µg /test.
Protocol
The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1% PBST for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.



Paraformaldehyde-fixed, paraffin embedded (Mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-MLK3(Thr277 + Ser281)) Polyclonal Antibody, Unconjugated (AP94722) at 1:500 overnight at 4°C, followed by a conjugated secondary (sp-0023) for 20 minutes and DAB staining.



Blank control: A431. Primary Antibody (green line): Rabbit Anti-MLK3(Thr277 + Ser281) antibody (AP94722) Dilution: 3 µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody: Goat anti-rabbit IgG-AF647 Dilution: 3 µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1% PBST for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.



Blank control (Black line): A431 (Black). Primary Antibody (green line): Rabbit Anti-MLK3 antibody (AP94722) Dilution: 1 µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647 Dilution: 1 µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1% PBST for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.