Certificate of Analysis MAPK14 (p38 alpha), Inactive, 100 µg

Mitogen Activated Protein (MAP) Kinase 14, Inactive, GST-tagged

Part Number: PV3305

Lot Number: 2725248C

Immediate Storage: -80°C

Shipping Conditions: dry ice

Description:

Recombinant human full length protein, inactive, GST-tagged, expressed in *E. coli.*

Specific Activity:

Approximately 0.0% activity as compared to the active form of the kinase.

Concentration:

 $0.76\ \text{mg/mL}$ total protein as measured using the Bradford protein assay with BSA as a standard.

Calculated 11,200 nM.

Aliases:

p38, Mxi2, SAPK2A

Storage and Handling:

Store at -80°C. At first use, aliquot and store at -80°C to avoid multiple freeze-thaws. If properly stored at -80°C, this product is guaranteed for 6 months from date of purchase.

Storage Buffer:

20 mM Tris (pH 7.5), 150 mM NaCl, 0.5 mM EDTA, 0.01% Triton $^{\otimes}$ X–100, 2 mM DTT and 50% Glycerol.

QUALITY ASSURANCE

Activation Test:

The coupled MAPK14 activation assay uses active MAP2K6, mutant (PV3293) to phosphorylate MAPK14. The activated MAPK14 then phosphorylates myelin basic protein (MBP). The assay was set up in two stages: 1st MAPK14 phosphorylation by MAP2K6, mutant without ³²P-ATP; and 2nd myelin basic protein (MBP) phosphorylation by activated MAPK14 in the presence of ³²P-ATP. The basal activity of MAP2K14 was also assayed in the absence of MAP2K6, mutant.

Dilution Buffer:

50 mM Tris (pH 7.5), 0.01% NP-40, 0.1 mg/mL BSA, 2 mM DTT and 1 mM Na_3VO_4.

Assay Conditions:

Both the active and inactive enzymes were diluted in enzyme dilution buffer and the reaction was set up in two stages. In stage one, MAPK14, inactive (3.33 µg/mL final concentration) was incubated with MAP2K6, mutant (PV3293) (0.83 µg/mL final concentration) or with dilution buffer for basal MAPK14, inactive activity in 50 mM Tris (pH 7.5), 10 mM MgCl₂, 1 mM EGTA, 2 mM DTT, 0.02% NP-40, 200 µM ATP for 30 minutes at 30°C. In stage two, 5 µL of the above reaction mix was transferred to 25 µL of reaction mix containing 50 mM Tris (pH 7.5), 10 mM MgCl₂, 1 mM EGTA, 2 mM DTT, 0.02% NP-40, 500 µM ATP, 1 mg/mL MBP substrate per reaction and trace [32 P]-γ-ATP and incubated for 10 minutes at 30°C.

Gel Information for MAPK14 (p38 alpha), Inactive

Page Description: The SDS- PAGE and/or Native PAGE were run on 4-20% Tris-Glycine Novex™ gels (Catalog #: EC6025BOX).		kDa 220 — 160 — 120 — 90 —	1111
L ane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).		80 — 70 — 60 — 50 — 40 —	-
L ane 2: 0.5 µg	-	30 —	
L ane 3: 1.0 µg		25 —	
L ane 4: 2.5 μg	=	15	
L ane 5: 5.0 μg		10 —	_
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Purity:

85% as determined by a SDS-PAGE gel stained with SimplyBlue™ SafeStain.

Molecular Weight:

67.6 kDa. Calculated from the protein sequence(s).

Mass Spectrometry:

MAPK14 (p38 alpha), Inactive was subjected to proteolytic digest followed by mass spec analysis. The resulting MS/MS data verified MAPK14 (p38 alpha) identity by comparison against the amino acid sequence(s) of the recombinant protein.



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Certificate of Analysis

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_620581

* highlighted residues denote differences from the reference protein sequence(s).

Chevohn Joseph, Director, Quality

Date: 29/Jun/2023

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