

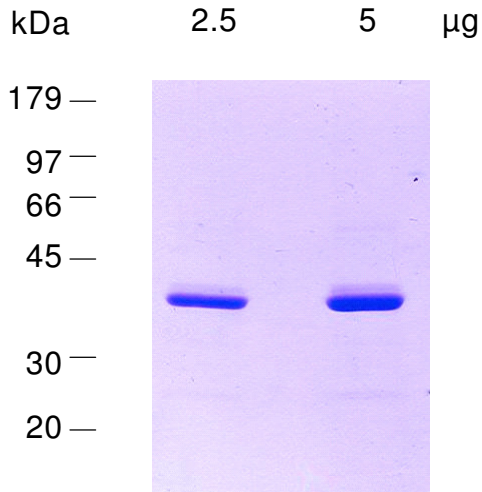
Certificate of Analysis

Product	p38alpha/ SAPK2A (MAPK14, isoform 2), active human recombinant, expressed in E.coli, N-His-fusion protein	
Cat No	PK-020-01	
Lot No	090307	
Description	Purified recombinant human p38alpha kinase (SAPK2A) expressed in E.coli. Active form of p38alpha kinase produced by phosphorylation of the purified p38alpha <i>in vitro</i> with MKK6. Phosphorylated at Thr180/Tyr182. Suitable for labeling p38alpha kinase substrates. Features a polyhistidine tag to facilitate removal of p38alpha kinase from the reaction mixture. Purified by Ni-agarose chromatography. Sequence based calculated M.W. 42,690. Approved HUGO gene symbol: MAPK14 Synonyms: mitogen activated protein kinase 14, PRKM14, CSBP2, p38, Mxi2, PRKM15, RK, EXIP, CSBP1, PRKM14, SAPK2a, p38alpha	
Quality	Protein concentration (Bradford with BSA as standard)	1.19 mg/ml
	Purity	> 95% by SDS PAGE
	Specific activity (* 1 Unit is defined as 1 picomole phosphate transferred to p38 peptide substrate RRRLVEPLTPSGEAPNQQ per min at 30 °C)	2,357,900 Units*/ mg
	Protease activity (Twining test)	none
Form	Liquid. In 25 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, 50 % glycerol, pH 8.5.	
Package size	20 microgram	
Storage condition	-70 °C	
Shipment conditions	dry ice	

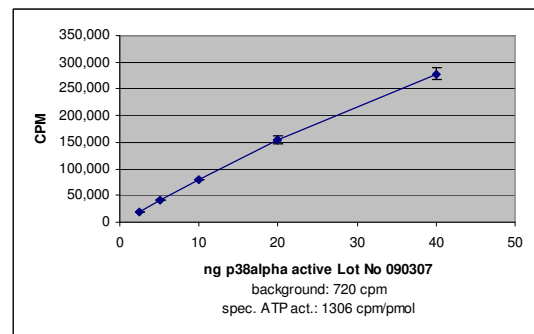
Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

SDS-PAGE analysis



Activity determination



Amino acid sequence information

M13 corresponds to M1 of L35253

MRGSHHHHHH	GSMSQERPTF	YRQELNKTIW	EVPERYQNLS	PVGSGAYGSV	50
CAAFDTKTGL	RVAVKKLSRP	FQSIIHAKRT	YRELRLKHM	KHENVIGLLD	100
VFTPARSLEE	FNDVYLVTHL	MGADLNNIVK	CQKLTDDHVQ	FLIYQILRGL	150
KYIHSADIIH	RDLKPSNLAV	NEDCELKILD	FGLARHTDDE	MTGYVATRWY	200
RAPEIMLNWM	HYNQTVDIWS	VGCIMAELLT	GRTLFPGTDH	IDQLKLILRL	250
VGTPGAELLK	KISSESARNY	IQSLTQMPKM	NFANVFIGAN	PLAVDLLEKM	300
LVLDSDKRIT	AAQALAHAYF	AQYHDPDDEP	VADPYDQSFE	SRDLLIDEWK	350
SLTYDEVISF	VPPPLDQEEM	ES			372

***In vitro* Kinase Assay**

Assay Components

Assay buffer (AB): 50 mM Tris-HCl, 10 mM NaCl, 10 mM DTT, pH 7.5

Substrate: p38 peptide substrate RRRLVEPLTPSGEAPNQK, 2 mM

Protein kinase: p38alpha, 4 ng/ microliter diluted in AB containing 0.1 % CHAPS directly before use

Magnesium/ATP Cocktail: 75 mM MgCl₂, 500 microM ATP

Diluted [γ -³²P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 microCi) [γ -³²P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig)

Assay procedure

All compounds are pipetted into a microcentrifuge tube on ice

1. Add 10 microliter AB
2. Add 10 microliter p38 peptide substrate RRRLVEPLTPSGEAPNQK, 2 mM
3. Add 10 microliter p38alpha (40 ng/assay)
4. Add 10 microliter of the diluted [γ -³²P]ATP
5. Incubate 10 min at 30 °C.
6. Stop the reaction by setting samples on ice
7. Remove 10 microliter and spot on P81 paper (let bind to the paper for 30 sec)
8. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
9. Wash 3 x with phosphoric acid
10. Wash 1 x with acetone
11. Dry under infrared light
12. Read in scintillation counter or Instant Imager