

ProQinase™ S6K

ribosomal protein S6 kinase B1

Recombinant Human Active Protein Kinase

HGNC Symbol: RPS6KB1

Synonyms: S6K1, STK14A, p70S6K, p70 S6K-alpha

Product No.: 0318-0000-2

Lot: 006

Description: Human S6K, internal fragment, amino acids M₂₄-K₄₂₃ (as in [NCBI/Protein](#) entry NP_003152.1), activated, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: S6K Lot 006, was confirmed as S6K by specific Western Blotting

Theoretical MW_{Fusion Protein}: 74,253 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: With PDK1

Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C

For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.422 µg/µl

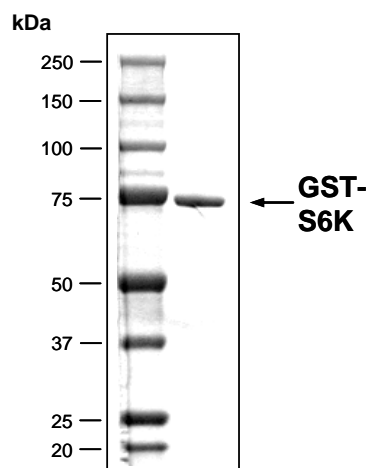
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 13 pmol/µg × min

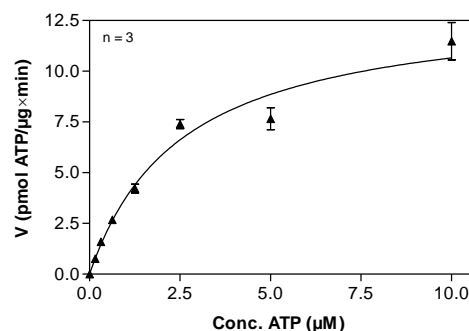
ATP-K_M: 2.6 µM

S6K Lot 006: Coomassie stain



2 µg GST-S6K

S6K Lot 006: Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:

- Assay conditions:
 - 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg/ml PEG_{20,000}
 - ATP (variable)
 - Substrate: S6-derived peptide 100 µg/ml
 - Kinase: 2 µg/ml
- Filter binding assay
- MSPH membrane (Millipore)

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GST-S6K Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH G	RRRASVAAGI	240
241	LVPRGS PGLD	GICSRMAGVF	DIDLQPEDA	GSEDELEEGG	QLNESMDHGG	VGPYELGMEH	300
301	CEKFEISETS	VNRGPEKIRP	ECFELLRLVLG	KGGYGKVFQV	RKVTGANTGK	IFAMKVLKKA	360
361	MIVRNAKDTA	HTKAERNILE	EVKHPFIVDL	IYAFQTGGKL	YLILEYLSGG	ELFMQLEREG	420
421	IFMEDTACFY	LAEISMALGH	LHQKGIYRD	LKPENIMLNH	QGHVKL TDFG	LCKESIHDGT	480
481	VTHTFCGTIE	YMAPEILMRS	GHNRAVDWWS	LGALMYDMLT	GAPPFTGENR	KKTIDKILKC	540
541	KLNLPPYLTQ	EARDLLKLL	KRNAASRLGA	GPGDAGEVQA	HPFFRHINWE	ELLARKVEPP	600
601	FKPLLQSEED	VSQFDSKFTR	QTPVDSPDDS	TLSESANQVF	LGFTYVAPSV	LESVK	660

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: S6K fragment

S6K wt ¹ Amino Acid Sequence							
1	MRRRRRRDGF	YPAPDFRDRE	AEDMAGVFDI	DLDQPEDAGS	EDELEEGGQL	NESMDHGGVG	60
61	PYELGMEHCE	KFEISETSVN	RGPEKIRPEC	FELLRLVLGK	GYGKVFQVRK	VTGANTGKIF	120
121	AMKVLKKAMI	VRNAKDTAHT	KAERNILEEV	KHPFIVDLIY	AFQTGGKLYL	ILEYLSGGEL	180
181	FMQLEREGIF	MEDTACFYLA	EISMALGHLH	QKGIIYRDLK	PENIMLNHQG	HVKL TDFGLC	240
241	KESIHDGTVT	HTFCGTIEYM	APEILMRS GH	NRAVDWWSLG	ALMYDMLTGA	PPFTGENRKK	300
301	TIDKILKCKL	NLPPYL TQEA	RDLLKLLKR	NAASRLGAGP	GDAGEVQAHP	FFRHINWEEL	360
361	LARKVEPPFK	PLLQSEEDVS	QFDSKFTRQT	PVDSPDDSTL	SESANQVFLG	FTYVAPSVLE	420
421	SVKEKFSFEP	KIRSPRRFIG	SPRTPVSPVK	FSPGDFWGRG	ASASTANPQT	PVEYPMETSG	480
481	IEQMDVTMSG	EASAPLPIRQ	PNSGPYKKA	FPMISKRPEH	LRMNL		540

blue: S6K sequence expressed in recombinant protein

¹[NCBI/Protein](#) accession number NP_003152.1