

ProQinase™ RB-S6P

Recombinant Protein Kinase Substrate

Product No.: 0339-0000-5

Lot: 021

Description: Artificial fusion protein RB-S6P consisting of a C-terminal fragment of human retinoblastoma protein RB1 ([NCBI/Protein entry NM_000312.2](#), aa S773-K928) and a peptide sequence (IAKRRRLSSLRASTSKSESSQK) derived from the human RPS6 protein ([NCBI/Protein entry NP_001001.2](#), aa I228-K249), N-terminal GST fusion protein with a Thrombin cleavage site, expressed in E.coli

Theoretical MW_{Fusion Protein}: 48,584 Da

Expression host: E.coli

Purification: GST-Affinity Chromatography

ATPase activity: In an ADP-Glo™ assay (Promega) with 10 µM ATP or 30 µM ATP, the ATP → ADP conversion within 30 min is approx. 1% at a concentration of 100 µg/ml substrate.

Detailed ATPase assay conditions on request

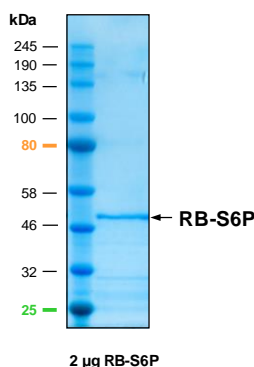
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

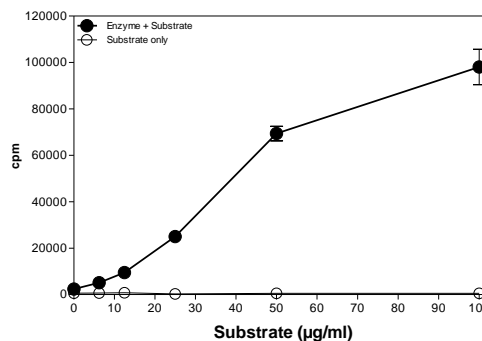
Protein concentration: 0.404 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

RB-S6P Lot 021:
Coomassie stain



Phosphorylation of RB-S6P by MYLK2

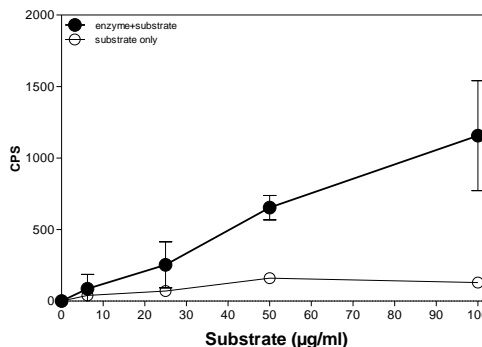
Radiometric filter binding assay



Assay conditions:

70 mM HEPES-NaOH, pH 7.5
3 mM MgCl₂
3 mM MnCl₂
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml PEG20.000
ATP: 1 µM
Substrate: variable concentration
MYLK2: 2 µg/ml
MSFC membrane (Millipore)

ADP-Glo™ assay (Promega)



70 mM HEPES-NaOH, pH 7.5
3 mM MgCl₂
3 mM MnCl₂
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml PEG20.000
ATP: variable concentration
1 % (v/v) DMSO
Substrate: variable concentration µg/ml
MYLK2: 2 µg/ml

This product was manufactured at Reaction Biology in Freiburg, Germany, and is for in vitro research use only, not for use in humans or animals.
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RB-S6P 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	LVPRGSP	TRPPTLSPIP	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRILV	SIGESFGTSE	300
301	KFQKINQMC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLP	ESKFQQLAE	360
361	MTSTRTRMQK	QKMNDSDTS	NKEEKI AKRR	RLSSLRASTS	KSESSQK		420

1-218: GST **Pink**: Thrombin cleavage site **blue**: RB1 fragment **boxed**: S6P sequence