

ProQinase™ RB-S6P

Recombinant Protein Kinase Substrate

Product No.: 0339-0000-5

Lot: 029

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 < 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, 2 mM DTT, 15 mM reduced glutathione, 10 % glycerol

Storage temperature: -80°C

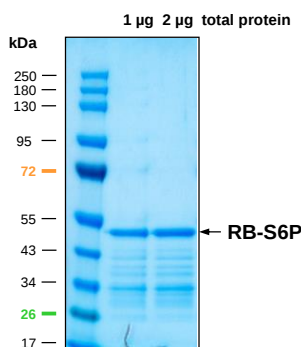
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10 µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.642 µg/µl

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

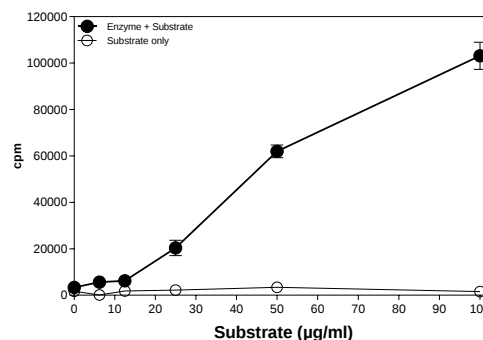
Substrate Lot 029:

Coomassie stain



Phosphorylation of RB-S6P by MYLK2

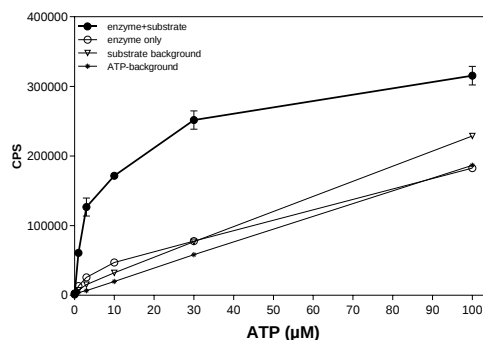
Radiometric filter binding assay



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 PEG_{20,000}
ATP: 1 µM
Substrate: variable concentration
Kinase: 2 µg/ml
FC filter assay (Corning)

ADP-Glo™ assay (Promega)



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 PEG_{20,000}
ATP: variable concentration
1 % (v/v) DMSO
Substrate: 80 µg/ml
Kinase: 2 µg/ml

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RB-S6P Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGDHPPKSD	LVPRGS PEFS	TRPPTLSPIP	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRIIV	SIGESFGTSE	300
301	KFQKINQMC	NSDRVLKRS	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLP	ESKFQQLAE	360
361	MTSTRTRMQK	QKMNDSDMTS	NKEEKIAKRR	RLSSLRASTS	KSESSQK		420

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