

# Certificate of Analysis

## RB-S6P

### Recombinant Protein Kinase Substrate

Product No.: 0339-0000-5

Lot: 012

**Description:** Artificial fusion protein RB-S6P consisting of a C-terminal fragment of human retinoblastoma protein RB1 (GenBank entry NM\_000321, aa S<sub>773</sub>-K<sub>928</sub>) and a peptide sequence (IAKRRRLSSLRASTSKSESSQK) derived from the human RPS6 protein (GenBank entry NM\_001010.2, aa I<sub>228</sub>-K<sub>249</sub>), N-terminal GST fusion protein with a Thrombin cleavage site, expressed in E.coli

**Theoretical MW<sub>Fusion Protein</sub>:** 46,584 Da

**Expression:** E.coli

**Purification:** Affinity chromatography using GSH-agarose, followed by ion chromatography

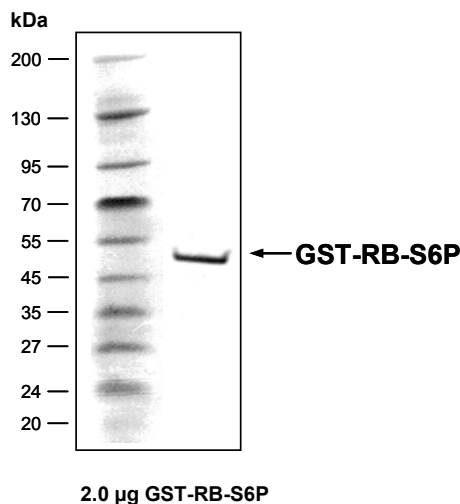
**ATPase activity:** In an ADP-Glo™ assay (Promega) with 10 μM ATP or 30 μM ATP, the ATP → ADP conversion within 30 min is below 1% at a concentration of 100 μg/ml RB-S6P\*.  
\*detailed ATPase assay conditions on request

**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 15 mM reduced glutathione, 10 % glycerol

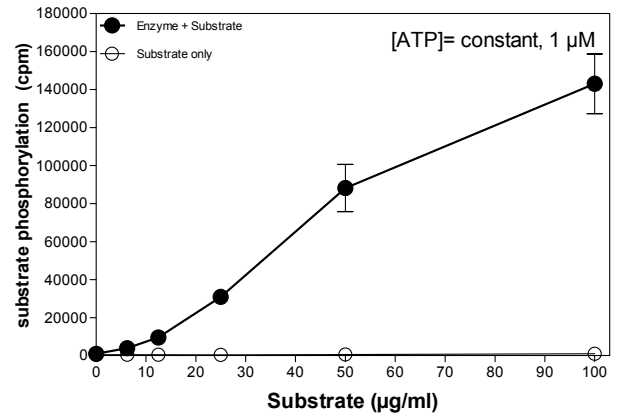
**Storage temperature:** -80°C  
Avoid repeated freeze-thaw cycles!

**Protein concentration:** 741 ng/μl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Coomassie stain:



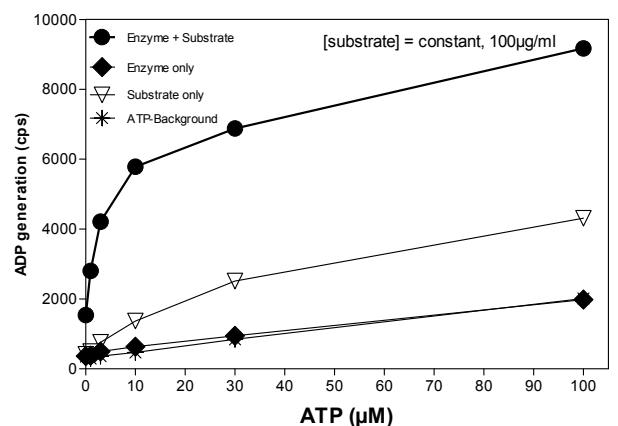
### Phosphorylation of RB-S6P by the kinase MYLK2 (radiometric filter binding assay):



Assay mixture:

70 mM HEPES-NaOH, pH 7.5  
3 mM MgCl<sub>2</sub>  
3 mM MnCl<sub>2</sub>  
3 μM Na-orthovanadate  
1.2 mM DTT  
50 μg/ml PEG<sub>20.000</sub>  
ATP: 1 μM  
Substrate (RB-S6P): variable concentration  
MYLK2: 2.0 μg/ml  
MSFC membrane (Millipore)

### Phosphorylation of RB-S6P by the kinase MYLK2 (ADP-Glo™ assay / Promega):



Assay mixture:

70 mM HEPES-NaOH, pH 7.5  
3 mM MgCl<sub>2</sub>  
3 mM MnCl<sub>2</sub>  
3 μM Na-orthovanadate  
1.2 mM DTT  
50 μg/ml PEG<sub>20.000</sub>  
ATP: variable concentration  
1 % (v/v) DMSO  
Substrate (RB-S6P): 100 μg/ml  
MYLK2: 2.0 μg/ml

This product is for in vitro research use only, not for use in humans or animals. ProQinase disclaims any warranty explicitly or implied that the use of the product or parts of the product is free from third party intellectual property claims unless this is explicitly stated.

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## RB-S6P

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RB-S6P Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTLL	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	WPLQGQWATF	GGGDHPPKSD	<b>LVPRGS</b> PEFS	<b>TRPPTLSPIP</b>	240
241	<b>HIPRSPYKFP</b>	<b>SSPLRIPGGN</b>	<b>IYISPLKSPY</b>	<b>KISEGLPTPT</b>	<b>KMTPRSRILV</b>	<b>SIGESFGTSE</b>	300
301	<b>KFQKINQMC</b>	<b>NSDRVLKRSA</b>	<b>EGSNPPKPLK</b>	<b>KLRFDIEGSD</b>	<b>EADGSKHLPG</b>	<b>ESKFQOKLAE</b>	360
361	<b>MTSTRTRMQK</b>	<b>QKMND SMDTS</b>	<b>NKEEKIAKRR</b>	<b>RLSSLRASTS</b>	<b>KSESSQK</b>		420

1-218: GST **Pink:** Thrombin **blue:**Rb fragment **shaded blue:** S6P peptide