

## RBBER-CHKtide

Recombinant Protein Kinase Substrate

Product No.: 0581-0000-5

Lot: 041

**Description:** Artificial fusion protein consisting of an N-terminal GST-tag separated by a Thrombin cleavage site from a fragment of the human retinoblastoma protein RB1, amino acids S<sub>773</sub>-K<sub>928</sub> (as in [NCBI/Protein](#) entry NP\_000312.2) followed by 11 Arg residues (ER) and a peptide (CHKtide) sequence KKKVSRSGLYRSPMPENLNRPR.

Expressed in E.coli.

**Theoretical MW<sub>Fusion Protein</sub>:** 48,526 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

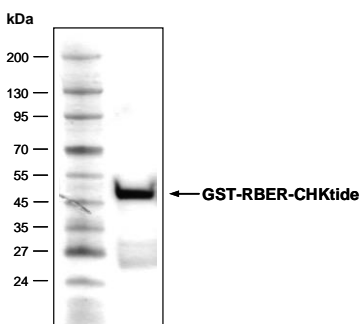
**Storage temperature:** -80°C

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

**Protein concentration:** 0.460 μg/μl (Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**RBBER-CHKtide Lot 041:**

**Coomassie stain**

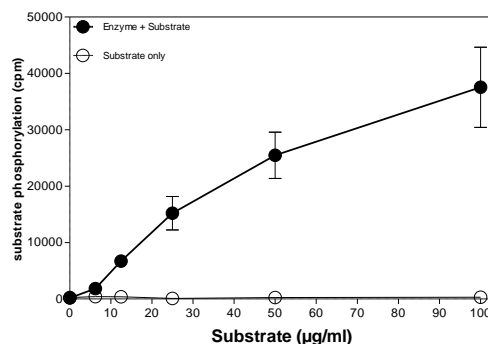


2.0 μg GST-RBER-CHKtide

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### Phosphorylation of RBBER-CHKtide by ERK2

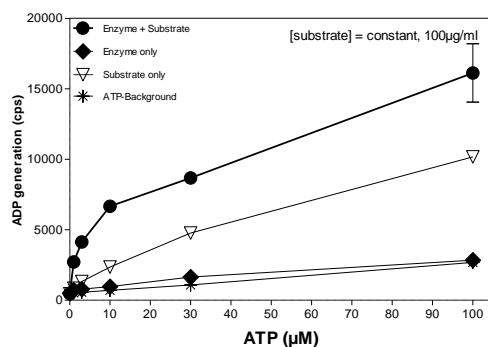
#### Radiometric filter binding assay



**Assay conditions:**

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: variable concentration  
 Kinase: 0.8 μg/ml  
 MSFC membrane (Millipore)

#### ADP-Glo™ assay (Promega)



**Assay conditions:**

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: 100 μg/ml  
 Kinase: 0.8 μg/ml

## Substrate

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SUBSTRATE Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	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	<b>LVPRGSP EFS</b>	<b>TRPPTLSPI P</b>	240
241	<b>HIPRSPYKFP</b>	<b>SSPLRIPGGN</b>	<b>IYISPLKSPY</b>	<b>KISEGLPTPT</b>	<b>KMTPRSRILV</b>	<b>SIGESFGTSE</b>	300
301	<b>KFQKINQMCV</b>	<b>NSDRVLKRSA</b>	<b>EGSNPPKPLK</b>	<b>KLRFDIEGSD</b>	<b>EADGSKHLPG</b>	<b>ESKFQQLAE</b>	360
361	<b>MTSTRTRMQK</b>	<b>QKMNDSMDTS</b>	<b>NKEEKRRRRR</b>	<b>RRRRR</b>	<b>KKKV</b>	<b>SRSGLYRSPS</b>	420

1-218: GST **Pink:** Thrombin cleavage site **Green:** R<sub>11</sub>-sequence **blue:** RB1 fragment **boxed:** CHKtide sequence