

# Certificate of Analysis



## RBER-CHKtide

Recombinant Protein Kinase Substrate

Product No.: 0581-0000-5

Lot: 041

**Description:** Artificial fusion protein consisting of a 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:** E.coli

**Purification:** Affinity chromatography using GSH-agarose, followed by ion exchange 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 RBER-CHKtide\*.  
\*detailed ATPase assay conditions on request

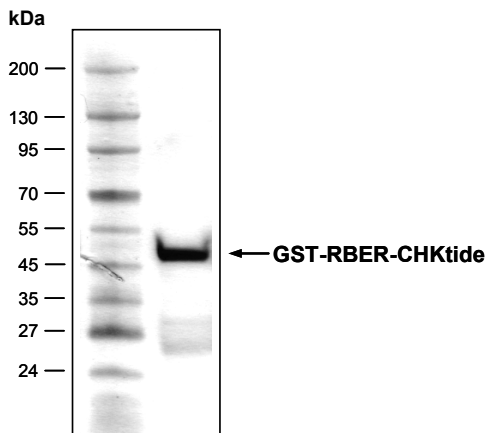
**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 15 mM reduced glutathione, 10% glycerol

**Storage temperature:** -80°C

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!

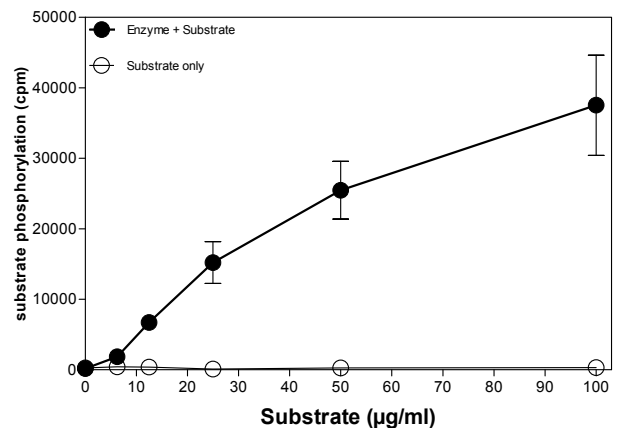
**Protein concentration:** 0.460 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Coomassie stain:**



2.0 µg GST-RBER-CHKtide

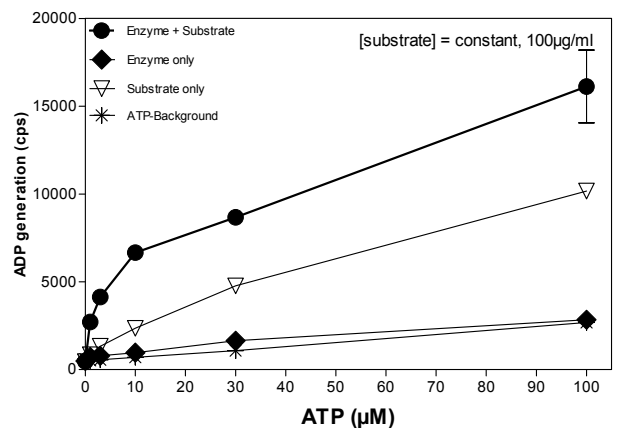
### Phosphorylation of RBER-CHKtide by the kinase ERK2 (Radiometric filter binding assay):



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 (RBER-CHKtide): variable concentration  
ERK2: 800 ng/ml  
MSFC membrane (Millipore)

### Phosphorylation of RBER-CHKtide by the kinase ERK2 (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 (RBER-CHKtide): 100 µg/ml  
ERK2: 800 ng/ml

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## RBBER-CHKtide

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RBBER-CHKtide 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	<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>NKEEKRRRRR</b>	<b>RRRRR</b> <b>KKKV</b>	<b>SRSGLYRSPS</b>	<b>MPENLNRPR</b>	420

1-218: GST   **Pink:** Thrombin cleavage site   **blue:** RB fragment   **green:** R11-tag   **shaded blue:** CHKtide peptide