

RBER-CDC25tide

Recombinant Protein Kinase Substrate

Product No.: 0590-0000-1

Lot: 004

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 S₇₇₃-K₉₂₈ (as in NCBI/Protein entry NP_000312.2) followed by 11 Arg residues (ER) and a peptide sequence ISDELMDATFADQEAK (CDC25tide). Expressed in E.coli.

Theoretical MW_{Fusion Protein}: 47,608 Da

Expression: E.coli

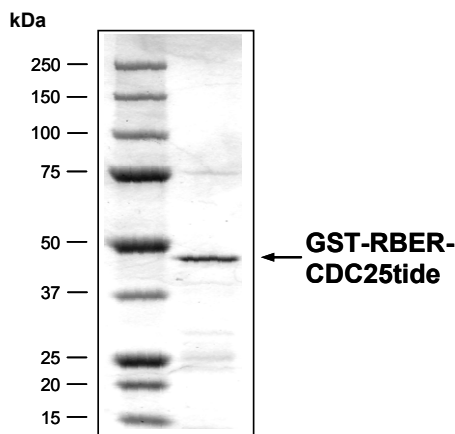
Purification: Affinity chromatography using GSH-agarose

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!

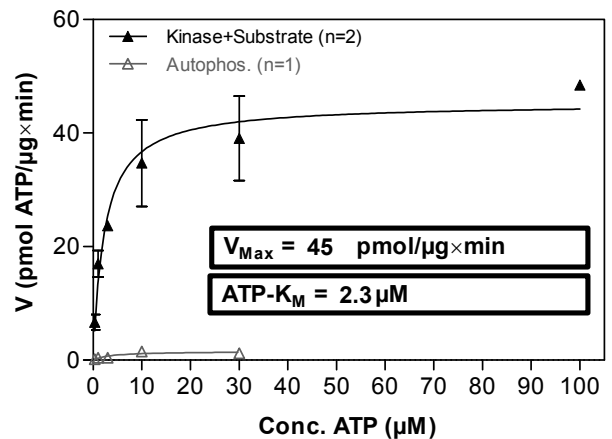
Protein concentration: 0.204 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:



2.0 µg GST-RBER-CDC25tide

Phosphorylation of RBER-CDC25tide by the kinase PLK1 (Radiometric filter binding assay):



Assay mixture:

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 (RBER-CDC25tide): variable concentration
PLK1: 1.0 µg/ml
MSFC membrane (Millipore)

Field of application:

RBER-CDC25tide has been validated for use in radiometric in-vitro kinase activity assays. It is not successfully validated for use in ATP-consumption based kinase activity assays.

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RBBER-CDC25tide Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSPEFS	TRPPTLSPIP	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRILV	SIGESFGTSE	300
301	KFQKINQMC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLPG	ESKFQQKLAE	360
361	MTSTRTRMQK	QKMNDSMDTS	NKEEKRRRRR	RRRRRISDELMDATFADQEA			420

1-218: GST **PINK: Thrombin** **blue:Rb fragment** **shaded green: R11-tag** **shaded red: PLK1S peptide**

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