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Certificate of Analysis

CRIK, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 16-040, 16-040-K, 16-040M
Parent Lot # D17HP011N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal GST-tagged, recombinant, human CRIK amino acids 1-449 expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.
Purity 88% by SDS-PAGE and Coomassie blue staining. MW = 77kDa.

Specific Activity (Parent lot# D17HP011N): 96U/mg, where one unit of CRIK activity is defined as 1nmol phosphate incorporated into 150µM KKLRRTLSVA per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.24mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

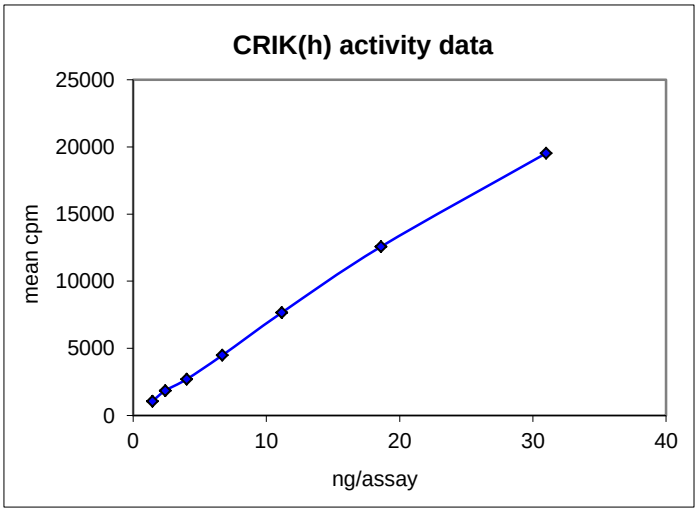
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

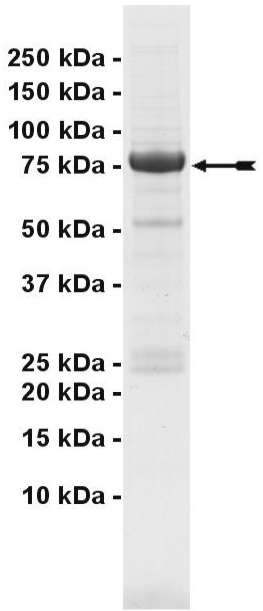
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1–31ng of this lot of enzyme phosphorylated 150µM KKLRRTLSVA in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as CRIK with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of CRIK, active..

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **KKLRRTLSVA**: Use at a final assay concentration of 150µM. Prepare a 1.5mM stock and add 2.5µl of stock per assay point.
3. **CRIK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–31ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[γ^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]ATP (specific activity approximately 1500 - 2400cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of KLRRTLSVA.
3. Add **2.5µl (1–31ng) CRIK, active.**
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 30 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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CRIK, active Sequence Information

Protein	Human CRIK
Tags	*N*-terminal GST
Native sequence	M230 of the recombinant protein is equivalent to M1 of human CRIK
Accession number	GenBank NM_007174.2

Recombinant CRIK amino acid sequence:

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   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKELM LKFKYGARNP
 241 LDAGAAEPIA SRASRLNLFF QGKPPFMTQQ QMSPLSREGI LDALFVLFEE CSQPALMKIK
 301 HVSNFVRKYS DTIAELQELQ PSAKDFEVRS LVGCGHFAEV QVVREKATGD IYAMKVMKKK
 361 ALLAQEQVSF FEEERNILSR STSPWIPQLQ YAFQDKNHLY LVMEYQPGGD LLSLLNRYED
 421 QLDENLIQFY LAELILAVHS VHLMGYVHRD IKPENILVDR TGHIKLVDFG SAAKMNSNKM
 481 VNAKLPIGTP DYMAPEVLTV MNGDGKGTYG LDCDWWSVGV IAYEMIYGRS PFAEGTSART
 541 FNNIMNFQRF LKFPDDPKVS SDFLDLIQSL LCGQKERLKF EGLCCHPFFS KIDWNNIRNS
 601 PPPFVPTLKS DDDTSNFDEP EKNSWVSSSP CQLSPSGFSG EELPFVGFSY SKALGILGRS
 661 ESVVSGLDSP AKTSSMEK
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Recombinant CRIK nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
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1261 cagttagatg aaaacctgat acagttttac ctagctgagc tgattttggc tgttcacagc
1321 gttcatctga tgggatacgt gcatcgagac atcaagcctg agaacattct cgttgaccgc
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1561 attgcctatg agatgattta tgggagatcc cccttcgcag agggaacctc tgccagaacc
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1621 ttcaataaca ttatgaattt ccagcggttt ttgaaatttc cagatgaccc caaagtgagc
1681 agtgactttc ttgatctgat tcaaagcttg ttgtgcggcc agaaagagag actgaagttt
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Reviewed and approved by site quality representative.