

Certificate of Analysis

eEF-2K, active

(Recombinant enzyme expressed in *E. coli* cells)

Item # 14-654, 14-654-K, 14-654M

Parent Lot # D7KN001U

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 eEF-2K, amino acids 2-end, expressed in *E. coli* cells. Purified using glutathione agarose. Purity 83.4% by SDS-PAGE and Coomassie blue staining. MW = 108.5kDa.

Specific Activity (Parent lot# D7KN001U): 1050U/mg, where one unit of eEF-2K, active activity is defined as 1nmol phosphate incorporated into 300µM (RKKFGESEKTKTKEFL) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.805mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

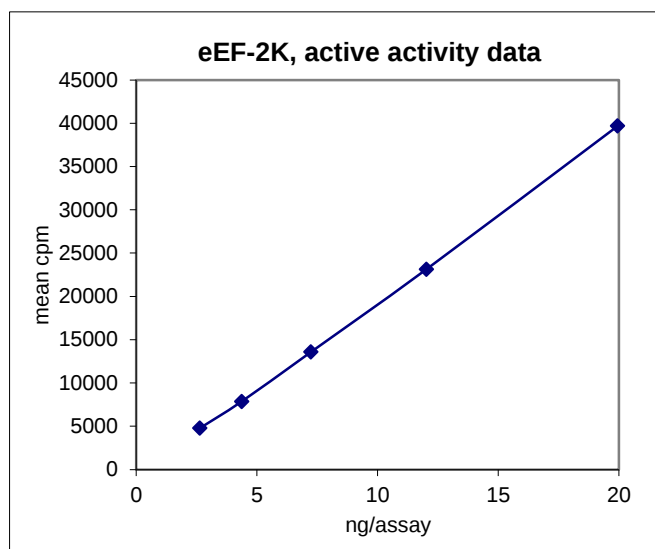
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

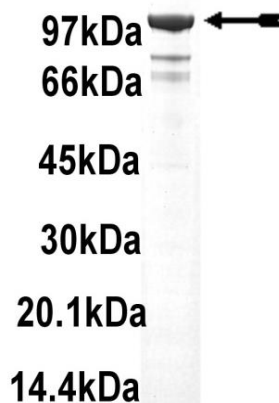
Quality Control Testing

Kinase Assay: 3–20ng of this lot of enzyme phosphorylated 300µM (RKKFGESEKTKTKEFL) 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 eEF-2K with the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of eEF-2K, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **CaCl₂:** Use at a final assay concentration of 500μM. Make a 5mM stock. Add 2.5μl of stock per assay point.
3. **(RKKFGESEKTKTKEFL):** Use at a final assay concentration of 300μM. Make a 3mM stock. Add 2.5μl of stock per assay point.
4. **Calmodulin:** Use at a final assay concentration of 0.016mg/ml. Make a 0.3mg/ml stock. Add 1.33μl of stock per assay point.
5. **eEF-2K active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3–20ng per assay point.
6. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/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 (**RKKFGESEKTKTKEFL**).
3. Add **2.5μl (3–20ng) eEf-2K, active**.
4. Add 2.5μl of 5mM CaCl₂.
5. Add 1.33μl of calmodulin.
6. Add 1.17μl of dH₂O.
7. Add 10μl of diluted [γ-³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5μl of 3% phosphoric acid.
10. Transfer a 10μl aliquot onto the appropriate area of a **P30 Filtermat**.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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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eEF-2K Sequence Information

<u>Protein</u>	Human eEF-2K
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	A227 of the recombinant protein is equivalent to A2 of human eEF-2K
<u>Accession number</u>	GenBank NM_013302. The recombinant protein contains the amino acid substitution H23R with reference to NM_013302. This conflict is reported in GenBank BC032665 and EST BM469975.

Recombinant eEF-2K amino acid sequence:

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1  MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQPID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSADED LIFRLEGVDG
241 GQSPRAGR DG DSDGSDDEE GYFICPITDD PSSNQNVNSK VNKYYSNLTK SERYS SSGSP
301 ANSFHFKEAW KHAIQKAKHM PDPWAEFHLE DIATERATRH RYNAV TGEWL DDEVLIKMAS
361 QPFGRGAMRE CFRTKKLSNF LHAQQWK GAS NYVAKRYIEP VDRDVYFEDV RLQMEAKLWG
421 EEYNRHKPPK QVDIMQMCII ELKDRPGKPL FHLEHYIEGK YIKYNSNSGF VRDDNIRLTP
481 QAFSHFTFER SGHQLIVVDI QGVGDLYTDP QIHTETGTDF GDGNLGV RGM ALFFYSHACN
541 RICESMGLAP FDLSPRERDA VNQNTKLLQS AKTILRGTEE KCGSPRVRTL SGRPPLLRP
601 LSENSGDENM SDVTFDSLPS SPSSATPHSQ KLDHLHWPVF SDLDNMA SRD HDHLDNHRES
661 ENSGDSGYPS EKRGE LDDPE PREHGHSYSN RKYSEDEDSL GSSGRVCVEK WNLLNSSRLH
721 LPRASAVALE VQRLNALDLE KKIGKSILGK VHLAMVRYHE GGRFCEKGEE WDQESAVFHL
781 EHAANLGELE AIVGLGLMYS QLP HHILADV SLKET EENKT KGFDYLLKAA EAGDRQSMIL
841 VARAFDSGQN LSPDRCDWL EALHWYNTAL EMTDCDEGGE YDGMQDEPRY MMLAREAEML
901 FTGGYGLEKD PQRS GDLYTQ AAEAA MEAMK GRLANQYYQK AEEAWAQ MEE
  
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Recombinant eEF-2K nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
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