

# University of Dundee

## Standard Operating Procedure

### Preparation of active EF2 Kinase [2 – 725]

**Enzyme description:-** EF2 Kinase [2 – 725]

**Clone number:-** DU 1446

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Expression level:-** 4 mg/L

**Calculated molecular mass:-** 108, 283 daltons

**Purity:-** 90 %

**Activation protocol:-** Constitutively active

**Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-** -70 °C

**Assay:-** Standard filter binding assay

**Assay buffer:-**

50 mM Hepes pH 6.6, 200  $\mu$ M CaCl<sub>2</sub>, 0.3  $\mu$ M calmodulin, 0.1 % 2-mercaptoethanol, 10 mM magnesium acetate

**Substrate:-**

RKKFGESEKTKTKEFL [Residues 2020 – 2035 of Dictyostelium myosin heavy chains]

Final concentration: 300  $\mu$ M

**Specific activity range:-** 150 – 300 U/mg

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## CLONE DATA SHEET - EF2 Kinase [2 – 725]

**Protein** EF2 Kinase [2 – 725]

**Clone number** DU 1446

**Species** Human

**Accession number** AAH32665

**Tags** N-terminal GST

**Bacterially expressed protein** MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA  
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKRIEAIPOIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDLVPRGSADEDLIFRLEGVDGGQSPR  
AGRDGSDGSDDEEGYFICPITDDPSSNQNVNSKVNKYYSNLTKSERY  
SSSGSPANSFHFKEAWKHAIQKAKHMPDPWAEFHLEDIATERATHRYN  
AVTGEWLDDEVLIKMASQPFGRGAMRECFRTKKLSNFLHAQQWKGASNY  
VAKRYIEPVDRDVYFEDVRLQMEAKLWGEEYNRHKPPKQVDIMQMCIE  
LKDRPGKPLFHLEHYIEGKYIKYNSNSGFVRRDDNIRLTPQAFSHFTFER  
SGHQLIVVDIQGVGDLYTDPQIHTETGTDFGDGNLGVRGMALFFYSHAC  
NRICESMGLAPFDLSPRERDAVNQNTKLLQSAKILRGTEEKCGSPRVR  
TLSGSRPPLLRPLSENSGDENMSDVTFDLSPSSPSSATPHSQKLDHLHW  
PVFSDLNMA SRDHDHLDNHRESENSGDSGYPSEKRGELDDPEPREHGH  
SYSNRKYESDEDSLGS SGRVCVEKWNLLNSSRLHLPRASAVALEVQRLN  
ALDLEKKIGKSILGKVHLAMVRYHEGGRFCEKGEEDQESAVFHLEHAA  
NLGELEAIVGLGLMYSQ LPHHILADVSLKETEENKTKGFDYLLKAAEAG  
DRQSMILVARAFDSGQNLSPDRCQDWLEALHWYNTALEMTDCDEGGEYD  
GMQDEPRYMMLAREAEMLFTGGYGLEKDPQRS GDLYTQAAEAAMEAMKG  
RLANQYYQKAE EAWAQMEE

**Native sequence** Amino acids A2 – E725 (end) of human EF2 kinase.  
Residue A227 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 - 220.

**Protease cleavage** Thrombin (LVPRGS) residues 221 - 226

**Cloning sites** BamH1 and EcoR1 site of pGEX 4T-1

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### Nucleotide Sequence of insert

atgtcccctatactaggttattggaaaattaagggccttgtgcaacca  
ctcgacttcttttggaaatcttgaagaaaaatatgaagagcatttgta  
tgagcgcgatgaaggtgataaatggcgaacaaaaagtttgaattgggt  
ttggagtttcccaatcttccttattatattgatgggtgatgttaaattaa  
cacagtctatggccatcatacgttatatagctgacaagcacaacatggt  
gggtggttgtccaaaagagcgtgcagagatttcaatgcttgaaggagcg  
gttttggatattagatacgggtgtttcgagaattgcatatagtaaagact  
ttgaaactctcaaagttgattttcttagcaagctacctgaaatgctgaa  
aatgttcgaagatcgtttatgtcataaaacatatttaaatgggtgatcat  
gtaacccatcctgacttcatgttgtatgacgctcttgatgttgttttat  
acatggaccaatgtgcctggatgcgttccaaaattagtttgttttaa  
aaaacgtattgaagctatcccacaaattgataagtacttgaaatccagc  
aagtatatagcatggcctttgcagggctggcaagccacgtttggtggtg  
gcgaccatcctccaaaatcggatctgggtccgcgtggatcc**GCAGACGA**  
**AGATCTCATCTTCCGCCTGGAAGGCGTTGATGGCGGCCAGTCCCCCGA**  
**GCTGGCCGTGATGGTGATTCTGATGGGGACAGCGACGATGAGGAAGGTT**  
**ACTTCATCTGCCCCATCACGGATGACCCAAGCTCGAACCAGAATGTCAA**  
**TTCCAAGGTTAATAAGTACTACAGCAACCTAACAAAAAGTGAGCGGTAT**  
**AGCTCCAGCGGGTCCCCGGCAAACCTCTTCCACTTCAAGGAAGCCTGGA**  
**AGCACGCAATCCAGAAGGCCAAGCACATGCCCCACCCCTGGGCTGAGTT**  
**CCACCTGGAAGATATTGCCACCGAACGTGCTACTCGACACAGGTACAAC**  
**GCCGTACCGGGGAATGGCTGGATGATGAAGTTCTGATCAAGATGGCAT**  
**CTCAGCCCTTCGGCCGAGGAGCAATGAGGGAGTGCTTCCGGACGAAGAA**  
**GCTCTCCAACCTTCTTGATGCCAGCAGTGGAAAGGGAGCCTCCAACCTAC**  
**GTGGCGAAGCGCTACATCGAGCCCGTAGACCGGGATGTGTACTTTGAGG**  
**ACGTGCGTCTACAGATGGAGGCCAAGCTCTGGGGGGAGGAGTATAATCG**  
**GCACAAGCCCCCAAGCAGGTGGACATCATGCAGATGTGCATCATCGAG**  
**CTGAAGGACAGACCGGGCAAGCCCCTCTTCCACCTGGAGCACTACATCG**  
**AGGGCAAGTACATCAAGTACAACCTCCAACCTCTGGCTTTGTCCGCGATGA**  
**CAACATCCGCCTGACCCGCAGGCCTTCAGCCACTTCACTTTTGAGCGT**  
**TCCGGCCATCAGCTGATAGTGGTGGACATCCAGGGAGTTGGGGATCTCT**  
**ACACTGACCCACAGATCCACACGGAGACGGGCACTGACTTTGGAGACGG**  
**CAACCTAGGTGTCCGCGGGATGGCGCTCTTCTTCTACTCTCATGCCTGC**  
**AACCGGATTTGCGAGAGCATGGGCCTTGCTCCCTTTGACCTCTCGCCCC**  
**GGGAGAGGGATGCAGTGAATCAGAACACCAAGCTGCTGCAATCAGCCAA**

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GACCATCTTGAGAGGAACAGAGGAAAAATGTGGGAGCCCCGAGTAAGG  
ACCCTCTCTGGGAGCCGGCCACCCCTGCTCCGTCGCCCTTTCAGAGA  
ACTCTGGAGACGAGAACATGAGCGACGTGACCTTCGACTCTCTCCCTTCTTC  
CCATCTTCGGCCACACCACACAGCCAGAAGCTAGACCACCTCCATTGG  
CCAGTGTTTCAGTGACCTCGATAACATGGCATCCAGAGACCATGATCATC  
TAGACAACCACCGGGAGTCTGAGAATAGTGGGGACAGCGGATACCCAG  
TGAGAAGCGGGGTGAGCTGGATGACCCTGAGCCCCGAGAACATGGCCAC  
TCATACAGTAATCGGAAGTACGAGTCTGACGAAGACAGCCTGGGCAGCT  
CTGGACGGGTATGTGTAGAGAAGTGGAAATCTCCTCAACTCCTCCCGCCT  
CCACCTGCCGAGGGCTTCGGCCGTGGCCCTGGAAGTGCAAAGGCTTAAT  
GCTCTGGACCTCGAAAAGAAAATCGGGAAGTCCATTTTGGGGAAGGTCC  
ATCTGGCCATGGTGCGCTACCACGAGGGTGGGCGCTTCTGCGAGAAGGG  
CGAGGAGTGGGACCAGGAGTCGGCTGTCTTCCACCTGGAGCACGCAGCC  
AACCTGGGCGAGCTGGAGGCCATCGTGGGCCTGGGACTCATGTACTCGC  
AGTTGCCTCATCACATCCTAGCCGATGTCTCTCTGAAGGAGACAGAAGA  
GAACAAAACCAAAGGATTTGATTACTTACTAAAGGCCGCTGAAGCTGGC  
GACAGGCAGTCCATGATCCTAGTGGCGCGAGCTTTTGACTCTGGCCAGA  
ACCTCAGCCCGGACAGGTGCCAAGACTGGCTAGAGGCCCTGCACTGGTA  
CAACACTGCCCTGGAGATGACGGACTGTGATGAGGGCGGTGAGTACGAC  
GGAATGCAGGACGAGCCCCGGTACATGATGCTGGCCAGGGAGGCCGAGA  
TGCTGTTACAGGAGGCTACGGGCTGGAGAAGGACCCGCAGAGATCAGG  
GGACTTGTATACCCAGGCAGCAGAGGCAGCGATGGAAGCCATGAAGGGC  
CGACTGGCCAACCAGTACTACCAAAGGCTGAAGAGGCCTGGGCCCAGA  
TGGAGGAGtaagaattc