Division of Signal Tranduction Therapy

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 µM $CaCl_2$, 0.3 µM calmodulin, 0.1 % 2−mercaptoethanol,
10 mM magnesium acetate

Substrate:-
RKKFGESEKTKTKEFL [Residues 2020 – 2035 of Dictyostelium myosin heavy chains]
Final concentration: 300 µM

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 LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLVPRGS**ADEDLIFRLEGVDGGQSPR** **AGRDGDSDGDSDDEEGYFICPITDDPSSNQNVNSKVNKYYSNLTKSERY** **SSSGSPANSFHFKEAWKHAIQKAKHMPDPWAEFHLEDIATERATRHRYN** **AVTGEWLDDEVLIKMASQPFGRGAMRECFRTKKLSNFLHAQQWKGASNY** **VAKRYIEPVDRDVYFEDVRLQMEAKLWGEEYNRHKPPKQVDIMQMCIIE** **LKDRPGKPLFHLEHYIEGKYIKYNSNSGFVRDDNIRLTPQAFSHFTFER** **SGHQLIVVDIQGVGDLYTDPQIHTETGTDFGDGNLGVRGMALFFYSHAC** **NRICESMGLAPFDLSPRERDAVNQNTKLLQSAKTILRGTEEKCGSPRVR** **TLSGSRPPLLRPLSENSGDENMSDVTFDSLPSSPSSATPHSQKLDHLHW** **PVFSDLDNMASRDHDHLDNHRESENSGDSGYPSEKRGELDDPEPREHGH** **SYSNRKYESDEDSLGSSGRVCVEKWNLLNSSRLHLPRASAVALEVQRLN** **ALDLEKKIGKSILGKVHLAMVRYHEGGRFCEKGEEWDQESAVFHLEHAA** **NLGELEAIVGLGLMYSQLPHHILADVSLKETEENKTKGFDYLLKAAEAG** **DRQSMILVARAFDSGQNLSPDRCQDWLEALHWYNTALEMTDCDEGGEYD** **GMQDEPRYMMLAREAEMLFTGGYGLEKDPQRSGDLYTQAAEAAMEAMKG** **RLANQYYQKAEEAWAQMEE**
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	*Bam*H1 and *Eco*R1 site of pGEX 4T-1

Nucleotide Sequence of insert

atgtcccctatactaggttattggaaaattaagggccttgtgcaaccca
ctcgacttcttttggaatatcttgaagaaaaatatgaagagcatttgta
tgagcgcgatgaaggtgataaatggcgaaacaaaaagtttgaattgggt
ttggagtttcccaatcttccttattatattgatggtgatgttaaattaa
cacagtctatggccatcatacgttatatagctgacaagcacaacatgtt
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gttttggatattagatacggtgtttcgagaattgcatatagtaaagact
ttgaaactctcaaagttgattttcttagcaagctacctgaaatgctgaa
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gtaacccatcctgacttcatgttgtatgacgctcttgatgttgttttat
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aaaacgtattgaagctatcccacaaattgataagtacttgaaatccagc
aagtatatagcatggcctttgcagggctggcaagccacgtttggtggtg
gcgaccatcctccaaaatcggatctggttccgcgtggatcc**GCAGACGA**
AGATCTCATCTTCCGCCTGGAAGGCGTTGATGGCGGCCAGTCCCCCCGA
GCTGGCCGTGATGGTGATTCTGATGGGGACAGCGACGATGAGGAAGGTT
ACTTCATCTGCCCCATCACGGATGACCCAAGCTCGAACCAGAATGTCAA
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ACGTGCGTCTACAGATGGAGGCCAAGCTCTGGGGGGAGGAGTATAATCG
GCACAAGCCCCCCAAGCAGGTGGACATCATGCAGATGTGCATCATCGAG
CTGAAGGACAGACCGGGCAAGCCCCTCTTCCACCTGGAGCACTACATCG
AGGGCAAGTACATCAAGTACAACTCCAACTCTGGCTTTGTCCGCGATGA
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TCCGGCCATCAGCTGATAGTGGTGGACATCCAGGGAGTTGGGGATCTCT
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GGGAGAGGGATGCAGTGAATCAGAACACCAAGCTGCTGCAATCAGCCAA

GACCATCTTGAGAGGAACAGAGGAAAAATGTGGGAGCCCCCGAGTAAGG
ACCCTCTCTGGGAGCCGGCCACCCCTGCTCCGTCCCCTTTCAGAGAACT
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GAACAAAACCAAAGGATTTGATTACTTACTAAAGGCCGCTGAAGCTGGC
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GGAATGCAGGACGAGCCCCGGTACATGATGCTGGCCAGGGAGGCCGAGA
TGCTGTTCACAGGAGGCTACGGGCTGGAGAAGGACCCGCAGAGATCAGG
GGACTTGTATACCCAGGCAGCAGAGGCAGCGATGGAAGCCATGAAGGGC
CGACTGGCCAACCAGTACTACCAAAAGGCTGAAGAGGCCTGGGCCCAGA
TGGAGGAGtaagaattc