

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active eIF2AK1 [1 - 630]

Enzyme description:- eIF2AK1 [1 - 630]

Clone number:- DU 67073

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 98, 241.96 daltons

Average Mass 98, 304.00 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.66

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2 mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

RSRSRSRSRSRSR residues 204 – 218 of human ASF-1/SF-2

Final concentration: 300 µM

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Clone Data Sheet

eIF2AK3 [1 - 600]

Protein eIF2AK3 [1 - 600]

Clone number DU 67073

Species Human

Accession number NM_014413.4

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPEFM**QGGNS**
GVRKREEEGDGAGAVAAPPAIDFPAEGPDPEYDESDVPAEIQVLKEPL
QOPTFPFAVANQLLLVSLLEHLSHVHEPNPLRSRQVFKLLCQTFIKMG
LLSSFTCSDEFSSRLHHNRAITHLMRSAKERV**RQDPCEDISRIQKIR**
SREVALEAQTSRYLNEFEELAILGKGGYGRVYKVRNKLDGQYYAIKKI
LIKGATKTVCMKVLREVKVLAGLQHPNIVGYHTAWIEHVHVIQPRADR
AAIELPSLEVLSDQEEDREQCGVKND**ESSSSSI**FAEPTPEKEKRFGE
SDTENQNNKSVKYTTNLVIRESGELESTLELQENGLAGLSASSIVE**QQ**
LPLRRNSHLEESFTSTEESSEENVN**FLGQTEAQYHLM**LHIQMQLCELS
LWDWIVERNKRGREYVDESACPYVMANVATKIFQELVEGVFYIHN**MG**I
VHRDLKPRNIFLHGPDQQVKIGDFGLACTDILQKNTDWTNRNGK**RTP**T
HTSRVGTCLYASPEQLEGSEYDAKSDM**YSLGVVLEL**FQPF**GTE**ME**R**A
EVL**TGLRTGQLPESLRKRC**PVQAKYIQHLTRRNSS**QRP**SAIQ**LLQ**SEL
FQNSGNVN**LTLMKIIEQEKEIAELKKQLN**LLS**QDKGVR**DDGKDGGVG

Native sequence Amino acids M1 – G630 (end) of human eIF2AK1.

Residue M235 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage Prescission site (LEVLFGQP) at residues 221 - 228

Cloning sites *Eco*R1 and *Not*I sites of pGEX-6P-1

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Nucleotide sequence of insert

gaattcATGCAGGGGGGCAACTCCGGGGTCCGCAAGCGCGAAGAGGAG
GGCGACGGGGCTGGGGCTGTGGCTGCGCCGCCGCCATCGACTTTCCC
GCCGAGGGCCCGGACCCCGAATATGACGAATCTGATGTTCCAGCAGAA
ATCCAGGTGTTAAAAGAACCCCTACAACAGCCAACCTTCCCTTTTGCA
GTTGCAAACCAACTCTTGCTGGTTCCTTGCTGGAGCACTTGAGCCAC
GTGCATGAACCAAACCCACTTCGTTC AAGACAGGTGTTTAAGCTACTT
TGCCAGACGTTTATCAAAATGGGGCTGCTGTCTTCTTTCACCTGTAGT
GACGAGTTTAGCTCATTGAGACTACATCACAACAGAGCTATTACTCAC
TTAATGAGGTCTGCTAAAGAGAGAGTTCGT CAGGATCCTTGTGAGGAT
ATTTCTCGTATCCAGAAAATCAGATCAAGGGAAGTAGCCTTGGAAGCA
CAAAC TTCAGTTACTTAAATGAATTTGAAGAACTTGCCATCTTAGGA
AAAGGTGGATACGGAAGAGTATACAAGGTCAGGAATAAATTAGATGGT
CAGTATTATGCAATAAAAAAATCCTGATTAAAGGTGCAACTAAAACA
GTTTG CATGAAGGTCCTACGGGAAGTGAAGGTGCTGGCAGGTCTTCAG
CACCCCAATATTGTTGGCTATCACACCGCGTGGATAGAACATGTTTCAT
GTGATTCAGCCACGAGCAGACAGAGCTGCCATTGAGTTGCCATCTCTG
GAAGTGCTCTCCGACCAGGAAGAGGACAGAGAGCAATGTGGTGTTAAA
AATGATGAAAGTAGCAGCTCATCCATTATCTTTGCTGAGCCCACCCCA
GAAAAAGAAAAACGCTTTGGAGAATCTGACACTGAAAAATCAGAATAAC
AAGTCGGTGAAGTACACCACCAATTTAGTCATAAGAGAATCTGGTGAA
CTTGAGTCGACCCTGGAGCTCCAGGAAAATGGCTTGGCTGGTTTGTCT
GCCAGTTCAATTGTGGAACAGCAGCTGCCACTCAGGCGTAATTCCCAC
CTAGAGGAGAGTTTTCACATCCACCGAAGAATCTTCCGAAGAAAATGTC
AACTTTTTTGGGTCAGACAGAGGCACAGTACCACCTGATGCTGCACATC
CAGATGCAGCTGTGTGAGCTCTCGCTGTGGGATTGGATAGTCGAGAGA
AACAAGCGGGGCCGGGAGTATGTGGACGAGTCTGCCTGTCTTATGTT
ATGGCCAATGTTGCAACAAAAATTTTTCAAGAATTGGTAGAAGGTGTG
TTTTACATACATAACATGGGAATTGTGCACCGAGATCTGAAGCCAAGA
AATATTTTTCTTCATGGCCCTGATCAGCAAGTAAAAATAGGAGACTTT
GGTCTGGCCTGCACAGACATCCTACAGAAGAACACAGACTGGACCAAC
AGAAACGGGAAGAGAACACCAACACATACGTCCAGAGTGGGTACTTGT
CTGTACGCTTCACCCGAACAGTTGGAAGGATCTGAGTATGATGCCAAG
TCAGATATGTACAGCTTGGGTGTGGTCCTGCTAGAGCTCTTTCAGCCG
TTTGGAACAGAAATGGAGCGAGCAGAAGTTCTAACAGGTTTAAGAACT
GGTCAGTTGCCGGAATCCCTCCGTAAAAGGTGTCCAGTGCAAGCCAAG
TATATCCAGCACTTAACGAGAAGGAACTCATCGCAGAGACCATCTGCC
ATTCAGCTGCTGCAGAGTGAACTTTTCCAAAATTCTGGAAATGTTAAC
CTCACCCCTACAGATGAAGATAATAGAGCAAGAAAAAGAAATTGCAGAA
CTAAAGAAGCAGCTAAACCTCCTTTCTCAAGACAAAGGGGTGAGGGAT
GACGGAAAGGATGGGGGCGTGGGAtgagcggccgc