MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active eIF2AK4 [1 - 1649]

Enzyme description:- eIF2AK4 [1 – 1649]

Clone number:- DU 67392

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 213, 600.17 daltons
Average Mass 213, 734.72 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.87

Purity:- >75 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70

Assay:- Standard filter binding assay

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

Substrate:-
RSRSRSRSRSRSRSR Final concentration: 300 uM

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Clone Data Sheet

eIF2AK4 [1 – 1649]

Protein	eIF2AK4 [1 – 1649]
Clone number	DU 67392
Species	Human
Accession number	NM_001013703.4
Tags	N-terminal GST

Native sequence

Amino acids M1 – F1649 (end) of human eIF2AK4.
Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage

PreScission (LEVLFQGP) residues 221 – 228

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MAGGRGAPG**
RGRDEPPESYPQRQDHELQALEAIYGADFQDLRPDACGPVKEPPEINL
VLYPQGLTGEEVYVKVDLRVKCPPTYPDVVPEIELKNAKGLSNESVNL
LKSRLEELAKKHCGEVMIFELAYHVQSFLSEHNKPPPKSFHEEMLERR
AQEEQQRLLEAKRKEEQEQREILHEIQRRKEEIKEEKKRKEMAKQERL
EIASLSNQDHTSKKDPGGHRTAAILHGGSPDFVGNGKHRANSSGRSRR
ERQYSVCNSEDSPGSCEILYFNMGSPDQLMVHKGKCIGSDEQLGKLVY
NALETATGGFVLLYEWVLQWQKKMGPFLTSQEKEKIDKCKKQIQGTET
EFNSLVKLSHPNVVRYLAMNLKEQDDSIVVDILVEHISGVSLAAHLSH
SGPIPVHQLRRYTAQLLSGLDYLHSNSVVHKVLSASNVLVDAEGTVKI
TDYSISKRLADICKEDVFEQTRVRFSDNALPYKTGKKGDVWRLGLLLL
SLSQGQECGEYPVTIPSDLPADFQDFLKKCVCLDDKERWSPQQLLKHS
FINPQPKMPLVEQSPEDSEGQDYVETVIPSNRLPSAAFFSETQRQFSR
YFIEFEELQLLGKGAFGAVIKVQNKLDGCCYAVKRIPINPASRQFRRI
KGEVTLLSRLHHENIVRYYNAWIERHERPAGPGTPPPDSGPLAKDDRA
ARGQPASDTDGLDSVEAAAPPPILSSSVEWSTSGERSASARFPATGPG
SSDDEDDDEDEHGGVFSQSFLPASDSESDIIFDNEDENSKSQNQDEDC
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WRLFREILDGLAYIHEKGMIHRDLKPVNIFLDSDDHVKIGDFGLATDH
LAFSADSKQDDQTGDLIKSDPSGHLTGMVGTALYVSPEVQGSTKSAYN
QKVDLFSLGIIFFEMSYHPMVTASERIFVLNQLRDPTSPKFPEDFDDG
EHAKQKSVISWLLNHDPAKRPTATELLKSELLPPPQMEESELHEVLHH
TLTNVDGKAYRTMMAQIFSQRISPAIDYTYDSDILKGNFSIRTAKMQQ
HVCETIIRIFKRHGAVQLCTPLLLPRNRQIYEHNEAALFMDHSGMLVM
LPFDLRIPFARYVARNNILNLKRYCIERVFRPRKLDRFHPKELLECAF
DIVTSTTNSFLPTAEIIYTIYEIIQEFPALQERNYSIYLNHTMLLKAI
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YKFIEQKGDLQDLMPTINSLIKQKTGIAQLVKYGLKDLEEVVGLLKKL
GIKLQVLINLGLVYKVQQHNGIIFQFVAFIKRRQRAVPEILAAGGRYD
LLIPQFRGPQALGPVPTAIGVSIAIDKISAAVLNMEESVTISSCDLLV
VSVGQMSMSRAINLTQKLWTAGITAEIMYDWSQSQEELQEYCRHHEIT
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RNGREASDNLAVQNLKGSFSNASGLFEIHGATVVPIVSVLAPEKLSAS
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ADEQAFNTTVKQLLSRLPKQRYLKLVCDEIYNIKVEKKVSVLFLYSYR
DDYYRILF

Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGGAA
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