

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active eIF2AK3 [536 – 1116]**

**Enzyme description:-** eIF2AK3 [536 - 1116]

**Clone number:-** DU 33545

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 93,477.28 daltons

Average Mass 93,537.10 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.64

**Purity:-** 85 %

**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 buffer:-**

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

**Substrate:-**

SRPKtide [RSRSRSRSRSRSRSR]

Final concentration: 300  $\mu$ M

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### Clone Data Sheet

#### eIF2AK3 [536 – 1116]

<b><u>Protein</u></b>	eIF2AK3 [536 - 1116]
<b><u>Clone number</u></b>	DU 33545
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_004836.5
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVD FLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSPNSRVDTTFIVRRL <b>FHPHPRQRKESETQCQTENKYDSVSGEANDSSWNDIKNSGYISRYLTD</b> <b>FEP IQCLGRGGFGVVF EAKNKVDDCNYAIKRIRLPNRELAREKVMREVK</b> <b>ALAKLEHPGIVRYFNAWLEAPPEKWQEKMDEIWLKDESTDWPLSSPSM</b> <b>DAPSVKIRMDPFSTKEHIEIIAPSPQRSRSFSVGISCDQTSSSESQFS</b> <b>PLEFSGMDHEDISESVDAAYNLQDSCLTDCDVEDGTMDGNDEGHSFELC</b> <b>PSEASPYVRSRERTSSSIVFEDSGCDNASSKEEPKTNRLHIGNHCANKL</b> <b>TAFKPTSSKSSSEATLSISPPRPTTSLDLTKNTTEKLQPS SPKVLYI</b> <b>QMQLCRKENLKDWMNGRCTIEERERSVCLHIFLQIAEAVEFLHSKGLMH</b> <b>RDLKPSNIFFTMDDVVKVGD FGLVTAMDQDEEEQTVLTPMPAYARHTGQ</b> <b>VGTKLYMSPEQIHGNSYSHKVDIFSLGLILFELLYPFSTQMERVRTLTD</b> <b>VRNLKFPPLFTQKYPCEYVMVQDMLSPSPMERPEAINI IENAVFEDLDF</b> <b>PGKTVLRQRSRLSSSGTKHSRQSNNSHSPLPSN</b></p>
<b><u>Native sequence</u></b>	Amino acids T536 – N1116 (end) of human eIF2AK3. Residue T238 of the fusion protein is equivalent to T536 of the native enzyme. The GST tag is located at residues 1 – 220.
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFQGP</u> ) residues 221 - 229
<b><u>Cloning sites</u></b>	<i>SalI</i> and <i>NotI</i> sites of pGEX 6P-1

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**Nucleotide**  
**Sequence of insert**

gtcgacACAACGTTTATTGTGCGCAGGCTTTTCCATCCTCATCCTCACA  
GGCAAAGGAAGGAGTCTGAAACTCAGTGTCAAACCTGAAAATAAATATGA  
TTCTGTAAGTGGTGAAGCCAATGACAGTAGCTGGAATGACATAAAAAAC  
TCTGGATATATATCACGATATCTAACTGATTTTGGAGCCAATTCAATGCC  
TGGGACGTGGTGGCCTTGGAGTTGTTTTTGAAGCTAAAAACAAAGTAGA  
TGACTGCAATTATGCTATCAAGAGGATCCGTCTCCCAATAGGGGAATTG  
GCTCGGGAAAAGGTAATGCGAGAAGTTAAAGCCTTAGCCAAGCTTGAAC  
ACCCGGGCATTGTTAGATATTTCAATGCCTGGCTCGAAGCACCACCAGA  
GAAGTGGCAAGAAAAGATGGATGAAATTTGGCTGAAAGATGAAAGCACA  
GACTGGCCACTCAGCTCTCCTAGCCCAATGGATGCACCATCAGTTAAAA  
TACGCAGAATGGATCCTTTCTCTACAAAAGAACATATTGAAATCATAGC  
TCCTTCACCACAAAAGAAGCAGGTCTTTTTTTCAGTAGGGATTTCTGTGAC  
CAGACAAGTTCATCTGAGAGCCAGTTCTCACCCTGGAATTTCTCAGGAA  
TGGACCATGAGGACATCAGTGAGTCAGTGGATGCAGCATAAACCTCCA  
GGACAGTTGCCTTACAGACTGTGATGTGGAAGATGGGACTATGGATGGC  
AATGATGAGGGGCACTCCTTTGAACTTTGTCCTTCTGAAGCTTCTCCTT  
ATGTAAGGTCAAGGGAGAGAACCTCCTCTTCAATAGTATTTGAAGATTC  
TGGCTGTGATAATGCTTCCAGTAAAGAAGAGCCGAAAACATAATCGATTG  
CATATTGGCAACCATTGTGCTAATAAACTAACTGCTTTCAAGCCCACCA  
GTAGCAAATCTTCTTCTGAAGCTACATTGTCTATTTCTCCTCCAAGACC  
AACCCTTTAAGTTTAGATCTCACTAAAAACACCACAGAAAAACTCCAG  
CCCAGTTCACCAAAGGTGTATCTTTACATTCAAATGCAGCTGTGCAGAA  
AAGAAAACCTCAAAGACTGGATGAATGGACGATGTACCATAGAGGAGAG  
AGAGAGGAGCGTGTGTCTGCACATCTTCTGCAGATCGCAGAGGCAGTG  
GAGTTTCTTACAGTAAAGGACTGATGCACAGGGACCTCAAGCCATCCA  
ACATATTCTTTACAATGGATGATGTGGTCAAGGTTGGAGACTTTGGGTT  
AGTACTGCAATGGACCAGGATGAGGAAGAGCAGACGGTTCTGACCCCA  
ATGCCAGCTTATGCCAGACACACAGGACAAGTAGGGACCAAACCTGTATA  
TGAGCCCAGAGCAGATTCATGGAAACAGCTATTCTCATAAAGTGGACAT  
CTTTTCTTTAGGCCTGATTCTATTTGAATTGCTGTATCCATTCAGCACT  
CAGATGGAGAGAGTCAGGACCTTAACTGATGTAAGAAATCTCAAATTTT  
CACCATTATTTACTCAGAAATATCCTTGTGAGTACGTGATGGTTCAAGA  
CATGCTCTCTCCATCCCCATGGAACGACCTGAAGCTATAAACATCATT  
GAAAATGCTGTATTTGAGGACTTGGACTTTCAGGAAAAACAGTGCTCA  
GACAGAGGTCTCGCTCCTTGAGTTCATCGGGAACAAAACATTCAGACA  
GTCCAACAACCTCCCATAGCCCTTTGCCAAGCAATtaggcggccgc