

## ***Division of Signal Transduction Therapy***

### **Standard Operating Procedure**

#### **Preparation of eIF4E [2 - 217]**

|                                           |                 |
|-------------------------------------------|-----------------|
| <b><u>Protein description:-</u></b>       | eIF4E [2 - 217] |
| <b><u>Clone number:-</u></b>              | DU 1129         |
| <b><u>Source:-</u></b>                    | Recombinant     |
| <b><u>Expression system:-</u></b>         | <i>E.coli</i>   |
| <b><u>Tag:-</u></b>                       | N-terminal GST  |
| <b><u>Purification method:-</u></b>       | GSH Sepharose   |
| <b><u>Expression level:-</u></b>          | 5 mg/L          |
| <b><u>Calculated molecular mass:-</u></b> | 51, 757 daltons |
| <b><u>Purity:-</u></b>                    | > 95 %          |

#### **Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2- mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

**Storage temperature:-** -20 °C

**Assay:-** Substrate for MNK2 alpha

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**CLONE DATA SHEET**

**eIF4E [2 – 217]**

|                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|---------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Protein</u></b>                       | eIF4E [2 - 217]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b><u>Clone number</u></b>                  | DU 1129                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b><u>Species</u></b>                       | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b><u>Accession number</u></b>              | NM_001968                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b><u>Tags</u></b>                          | N-terminal GST                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b><u>Bacterially expressed protein</u></b> | <p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL<br/>GLEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAIEISMLE<br/>GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN<br/>GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPIQIDKY<br/>LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGGPLGSATVEPETTP<br/>TPNPPTTEEEKTESNQEVANPEHYIKHPLQNRWALWFFKNDKSKTWQA<br/>NLRLISKFDTVEDFWALYNHIQLSSNLMPGCDYSLFKDGI EPMWEDEK<br/>NKRGGRWLITLNKQQRSDLD RFWLETLLCLIGESFDDYSDDVCGAVV<br/>NVRAKGDKIAIWTTECENREAVTHIGRVYKERLGLPPKIVIGYQSHAD<br/>TATKSGSTTKNRFV</p> |
| <b><u>Native sequence</u></b>               | <p>Amino acids A2 – V217 (end) of human eIF4E.<br/>Residue A232 of the fusion protein is equivalent to A2 of the native<br/>protein. The GST tag is located at residues 1 – 220</p>                                                                                                                                                                                                                                                                                                                                 |
| <b><u>Protease cleavage</u></b>             | PreScission ( <u>LEVLFQGPL</u> ) residues 221 - 229                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b><u>Cloning sites</u></b>                 | <i>Bam</i> HI and <i>Eco</i> RI sites of pGEX 6P-1                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

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### Nucleotide sequence of insert

GGATCCGCGACTGTCTGAACCGGAAACCAACCCCTACTCCTAA  
TCCCCGACTACAGAAGAGGAGAAAAACGGAATCTAATCAGG  
AGGTTGCTAACCCAGAACACTATATTAAACATCCCCTACAG  
AACAGATGGGCACTCTGGTTTTTTAAAAATGATAAAAGCAA  
AACTTGGCAAGCAAACCTGCGGCTGATCTCCAAGTTTGATA  
CTGTTGAAGACTTTTGGGCTCTGTACAACCATATCCAGTTG  
TCTAGTAATTTAATGCCTGGCTGTGACTACTCACTTTTTTAA  
GGATGGTATTGAGCCTATGTGGGAAGATGAGAAAAACAAAC  
GGGGAGGACGATGGCTAATTACATTGAACAAACAGCAGAGA  
CGAAGTGACCTCGATCGCTTTTGGCTAGAGACACTTCTGTG  
CCTTATTGGAGAATCTTTTGATGACTACAGTGATGATGTAT  
GTGGCGCTGTTGTTAATGTTAGAGCTAAAGGTGATAAGATA  
GCAATATGGACTACTGAATGTGAAAACAGAGAAGCTGTTAC  
ACATATAGGGAGGGTATACAAGGAAAGGTTAGGACTTCCTC  
CAAAGATAGTGATTGGTTATCAGTCCCACGCAGACACAGCT  
ACTAAGAGCGGCTCCACCACTAAAAATAGGTTTGTTGTTta  
agaattc