

# *MRC PPU Reagents and Services*

## **Standard Operating Procedure**

### **Preparation of eIF2S2 [1 – 333]**

**Enzyme description:-** eIF2S2 [1 - 333]

**Clone number:-** DU 68476

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 65, 171.01 daltons

Average Mass 65, 212.57 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 5.66

**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, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-** -70 °C

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## Clone Data Sheet

### eIF2S2 [1 - 333]

|                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Protein</u></b>                       | EIF2S2 [1 - 333]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b><u>Clone number</u></b>                  | DU 68476                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b><u>Species</u></b>                       | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b><u>Accession number</u></b>              | NM_003908.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b><u>Tags</u></b>                          | N-terminal GST                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b><u>Bacterially expressed protein</u></b> | <p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG<br/>LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA<br/>VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH<br/>VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS<br/>KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSM<b>SGDEMIFDPTMSK</b><br/><b>KKKKKKKPFMLDEEGDTQTEETQPSETKEVEPEPTEDKDLEADEEDTRK</b><br/><b>KDASDDLDDLNFFNQKKKKKKTKKIFDIDEAEEGVKDLKIESDVQEPTE</b><br/><b>PEDDLDIMLGNNKKKKKNVKFPDEDEILEKDEALEDEDNKKDDGISFSN</b><br/><b>QTGPAWAGSERDYTYEELLNRVFNIMREKNPDMVAGEKRKFVMKPPQVV</b><br/><b>RVGTTKTSFVNFTDICKLLHRQPKHLLAFLLAELGTSGSIDGNNQLVIK</b><br/><b>GRFQQKQIENVLRRYIKEYVTCHTCRSPDTILQKDTRL YFLQCETCHSR</b><br/><b>CSVASIKTGFQAVTGKRAQLRAKAN</b></p> |
| <b><u>Native sequence</u></b>               | <p>Amino acids M1 – N333 (end) of human eIF2S2.<br/>Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b><u>Protease cleavage</u></b>             | PreScission ( <u>LEVLFQGP</u> ) residues 221 - 229                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b><u>Cloning sites</u></b>                 | <i>Bam</i> HI and <i>Not</i> I site of pGex6P-1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

## *MRCPPU Reagents and Services*

### **Nucleotide Sequence of Insert**

ggatccATGTCTGGGGACGAGATGATTTTTGATCCTACTATGAGCAAGAAGAAAAAGAAGAAG  
AAGCCTTTTATGTTAGATGAGGAAGGGGATACCCAAACAGAGGAAACCCAGCCTTCAGAAACAAAA  
GAAGTGGAGCCAGAGCCAACTGAGGACAAGGATTTGGAAGCTGATGAAGAGGACACTAGGAAAAAA  
GATGCTTCTGATGATCTAGATGACTTGAACCTCTTTAATCAAAAAGAAAAAGAAGAAAAAACTAAA  
AAGATATTTGATATTGATGAAGCTGAAGAAGGTGTAAAGGATCTTAAGATTGAAAGTGATGTTCAA  
GAACCAACTGAACCAGAGGATGACCTTGACATTATGCTTGGCAATAAAAAGAAGAAAAAGAAGAAT  
GTTAAGTTCCTCAGATGAGGATGAAATACTAGAGAAAGATGAAGCTCTAGAAGATGAAGACAACAAA  
AAAGATGATGGTATCTCATTTCAGTAATCAGACAGGCCCTGCTTGGGCAGGCTCAGAAAGAGACTAC  
ACATACGAGGAGCTGCTGAATCGAGTGTTCAACATCATGAGGGAAAAAGAATCCAGATATGGTTGCT  
GGGGAGAAAAGGAAATTTGTCATGAAACCTCCACAAGTCGTCCGAGTAGGAACCAAGAAAACCTTCT  
TTTGTCAACTTTACAGATATCTGTAACTATTACATCGTCAGCCCAAACATCTCCTTGCAATTTTG  
TTGGCTGAATTGGGTACAAGTGGTTCTATAGATGGTAATAACCAACTTGTAATCAAAGGAAGATTC  
CAACAGAAACAGATAGAAAATGTCTTGAGAAGATATATCAAGGAATATGTCACTTGTCACACATGC  
CGATCACCGGACACAATCCTGCAGAAGGACACACGACTCTATTTCTACAGTGCGAACTTGTCAT  
TCTAGATGTTCTGTTGCCAGTATCAAAACCGGCTTCCAGGCTGTCACGGGCAAGCGAGCACAGCTC  
CGTGCCAAAGCTAACTaagcggccgc