

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of eIF2S1 [1 – 315]

Enzyme description:- eIF2S1 [1 - 315]

Clone number:- DU 68477

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 62, 896.01 daltons

Average Mass 62, 936.33 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.23

Purity:- >85 %

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

eIF2S1 [1 - 315]

| | |
|---|---|
| <u>Protein</u> | EIF2S1 [1 - 315] |
| <u>Clone number</u> | DU 68477 |
| <u>Species</u> | Human |
| <u>Accession number</u> | NM_004094.5 |
| <u>Tags</u> | N-terminal GST |
| <u>Bacterially expressed protein</u> | <p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSMPGLSCRFYQHKFP EVEDVVMVNVRSIAEMGAYVSLLEYNNIEGMILLSELSRRRIRSINKLI RIGRNECVVIRVDKEKGYIDLSKRRVSPEEAIKCEDKFTKSKTVYSIL RHVAEVLEYTKDEQLES LFQRTAWVFDDKYKRPGYGAYDAFKHAVSDPS ILDSL DLNEDEREVLINNI NRRLTPQAVKIRADIEVACYGYEGIDAVKE ALRAGLNCSTENMPIKINLIAPPRYVMTTTTLERTEGLSVLSQAMAVIK EKIEEKRGVFNVQMEPKVVTDTDET ELARQMERLERENA EVDGDDDAEE MEAKAED</p> |
| <u>Native sequence</u> | <p>Amino acids M1 – D315 (end) of human eIF2S1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p> |
| <u>Protease cleavage</u> | PreScission (<u>LEVLFQGP</u>) residues 221 - 229 |
| <u>Cloning sites</u> | <i>Bam</i> HI and <i>Not</i> I site of pGex6P-1 |

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Nucleotide Sequence of Insert

ggatccATGCCGGGTCTAAGTTGTAGATTTTATCAACACAAATTTCTGAGGTGGAAGATGTAGTGATGGTGAATGTCAGAT
CCATTGCTGAAATGGGGGCTTATGTCAGCTTGCTGGAATACAACAACATTGAAGGCATGATTCTTCTTAGTGAATTATCCA
GAAGGCGTATCCGTTCTATCAACAAACTCATCCGAATTGGCAGGAATGAGTGTGTGGTTGTCATTAGGGTGGACAAAGAA
AAAGGATATATTGATTTGTCAAAAAGAAGAGTTTCTCCAGAGGAAGCAATCAAATGTGAAGACAAATTCACAAAATCCAAA
ACTGTTTATAGCATTCTTCGTATGTTGCTGAGGTGTTAGAATACACCAAGGATGAGCAGCTGGAAAGCCTATTCCAGAGG
ACTGCCTGGGTCTTTGATGACAAGTACAAGAGACCTGGATATGGTGCCTATGATGCATTTAAGCATGCAGTCTCAGACCCA
TCTATTTTGGATAGTTTAGATTTGAATGAAGATGAACGGGAAGTACTCATTAAATAATTAATAGGCGCTTGACCCACAGG
CTGTCAAATTCGAGCAGATATTGAAGTGGCTTGTATGGTTATGAAGGCATTGATGCTGTAAAAGAAGCCCTAAGAGCAG
GTTTGAATTGTTCTACAGAAAACATGCCCATTAAGATTAATCTAATAGCTCCTCCTCGGTATGTAATGACTACGACAACCT
GGAGAGAACAGAAGGCCTTTCTGTCCTCAGTCAAGCTATGGCTGTTATCAAAGAGAAGATTGAGGAAAAGAGGGGTGTGT
TCAATGTTCAAATGGAGCCCAAAGTGGTCACAGATACAGATGAGACTGAACTTGCGAGGCAGATGGAGAGGCTTGAAAAGA
GAAAATGCCGAAGTGGATGGAGATGATGATGCAGAAGAAATGGAAGCCAAAGCTGAAGATtaagcggccgc