Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Eukaryotic Translation Initiation Factor 4E binding protein 1 [1 – 118]

Enzyme description:- 4E-BP1 [1 - 118]

Clone number:- DU 10998

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 39, 936.02 daltons
Average Mass 39, 961.81 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.51

Purity:- >80 %

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:- Substrate for mTOR

Division of Signal Tranduction Therapy

Clone Data Sheet

4E-BP1 [1 – 118]

Protein	4E-BP1 [1 - 118]
Clone number	DU 10998
Species	Human
Accession number	NM_004095.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEFAL**MSGGSSCSQ TPSRAIPATRRVVLGDGVQLPPGDYSTTPGGTLFSTTPGGTRIIYDRKF LMECRNSPVTKTPPRDLPTIPGVTSPSSDEPPMEASQSHLRNSPEDKRA GGEESQFEMDI**
Native sequence	Amino acids M1 – I118 (end) of human 4E-BP1. Residue M237 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 - 229
Cloning sites	*Bam*H1 and *Not*1sites of pGEX 6P-1

Nucleotide Sequence of insert

ggatccccggaattcgcccttATGTCCGGGGGCAGCAGCTGC
AGCCAGACCCCAAGCCGGGCCATCCCCGCCACTCGCCGGGTG
GTGCTCGGCGACGGCGTGCAGCTCCCGCCCGGGGACTACAGC
ACGACCCCCGGCGGCACGCTCTTCAGCACCACCCCGGGAGGT
ACCAGGATCATCTATGACCGGAAATTCCTGATGGAGTGTCGG
AACTCACCTGTGACCAAAACACCCCCAAGGGATCTGCCCACC
ATTCCGGGGGTCACCAGCCCTTCCAGTGATGAGCCCCCCATG
GAAGCCAGCCAGAGCCACCTGCGCAATAGCCCAGAAGATAAG
CGGGCGGGCGGTGAAGAGTCACAGTTTGAGATGGACATTtaa
aagggcgaattcccgggtcgactcgagcggccgc