Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Deptor [1 - 409]

Enzyme description:-	Deptor [1 – 409]
Clone number:-	DU 30399
Source:-	Recombinant
Expression system:-	*E.coli*,
Tag:-	N-terminal GST
Purification method:-	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	73, 070.93 daltons
Average Mass	73, 117.97 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	6.88
Purity:-	>80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:-	-70 °C

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Clone Data Sheet

Deptor [1 - 409]

Protein	Deptor [1 - 409]
Clone number	DU 30399
Species	Human
Accession number	NM_022783.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MEEGGSTGS** **AGSDSSTSGSGGAQQRELERMAEVLVTGEQLRLRLHEEKVIKDRRHHL** **KTYPNCFVAKELIDWLIEHKEASDRETAIKLMQKLADRGIIHHVCDEH** **KEFKDVKLFYRFRKDDGTFPLDNEVKAFMRGQRLYEKLMSPENTLLQP** **REEEGVKYERTFMASEFLDWLVQEGEATTRKEAEQLCHRLMEHGIIQH** **VSNKHPFVDSNLLYQFRMNFRRRRRLMELLNEKSPSSQETHDSPFCLR** **KQSHDNRKSTSFMSVSPSKEIKIVSAVRRSSMSSCGSSGYFSSSPTLS** **SSPPVLCNPKSVLKRPVTSEELLTPGAPYARKTFTIVGDAVGWGFVVR** **GSKPCHIQAVDPSGPAAAAGMKVCQFVVSVNGLNVLHVDYRTVSNLIL** **TGPRTIVMEVMEELEC**
Native sequence	Amino acids M1 – C409 (end) of human Deptor. Residue M232 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	Prescission site (LEVLFQGP) at residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1sites of pGex6P-1

Nucleotide sequence of insert

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ggatccATGGAGGAGGGCGGCAGCACTGGCAGTGCTGGCAGTGACAGC
AGCACCAGCGGGAGTGGCGGGGCGCAGCAAAGGGAGCTGGAGCGCATG
GCTGAGGTCTTGGTCACCGGGGAACAGCTACGGCTCAGGCTGCACGAA
GAAAAGGTTATTAAAGATAGACGTCATCATCTCAAGACCTACCCAAAC
TGTTTTGTCGCAAAAGAACTGATTGACTGGCTGATTGAACACAAAGAG
GCTTCTGACAGAGAGACGGCAATTAAACTCATGCAGAAATTAGCAGAC
CGGGGCATTATTCACCATGTGTGTGATGAGCATAAGGAATTCAAGGAT
GTCAAACTCTTCTACCGCTTTAGAAAGGATGACGGCACCTTCCCATTG
GATAATGAAGTGAAGGCCTTTATGAGAGGACAGAGGCTATATGAAAAG
CTGATGAGCCCTGAAAACACACTCCTGCAGCCCAGGGAGGAGGAAGGG
GTCAAGTATGAGCGCACCTTCATGGCATCTGAATTCCTGGACTGGCTG
GTTCAGGAAGGTGAGGCCACCACGAGGAAAGAGGCAGAGCAGCTTTGC
CACCGGCTTATGGAGCATGGCATCATCCAGCATGTGTCCAACAAGCAC
CCATTTGTGGACAGCAATCTTCTCTACCAGTTCAGAATGAACTTCCGG
CGGAGGCGAAGACTGATGGAGCTGCTCAATGAAAAGTCCCCCTCCTCC
CAGGAAACTCATGACAGTCCCTTCTGCCTGAGGAAGCAGAGCCATGAC
AATCGGAAATCTACCAGCTTTATGTCAGTGAGCCCCAGCAAGGAGATC
AAGATCGTGTCTGCAGTGAGGAGAAGCAGCATGAGCAGCTGTGGCAGC
AGCGGCTACTTCAGCAGCAGCCCCACCCTCAGCAGCAGCCCCCCCTGTG
CTCTGCAACCCCAAGTCCGTGCTGAAGAGACCTGTCACCTCTGAGGAA
CTCCTTACTCCCGGGGCTCCGTATGCAAGGAAGACATTCACGATTGTT
GGTGACGCGGTTGGCTGGGGTTTTGTGGTGCGAGGAAGTAAGCCATGC
CACATCCAGGCTGTAGACCCCAGTGGCCCTGCAGCCGCAGCAGGAATG
AAGGTCTGTCAGTTTGTCGTCTCTGTCAACGGGCTCAATGTCCTGCAT
GTAGACTACCGGACCGTGAGCAATCTGATTCTGACGGGCCCACGGACG
ATTGTCATGGAAGTCATGGAGGAGTTAGAGTGCtgagcggccgc
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