MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of M025 alpha [2 - 341]

Enzyme description:- MO25 alpha [2 – 341]

Clone number:- DU 30906

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and MYC

Purification method:- GSH Agarose

Calculated molecular mass:-
Monoisotopic 68, 007.06 daltons
Average Mass 68, 050.64 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.84

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

MO25 alpha [2 - 341]

Protein	MO25 alpha [2 - 341]
Clone number	DU 30906
Species	Human
Accession number	NM_016289.4
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSATM*EQKLIS* *EEDL***PFPFGKSHKSPADIVKNLKESMAVLEKQDISDKKAEKATEEVSK** **NLVAMKEILYGTNEKEPQTEAVAQLAQELYNSGLLSTLVADLQLIDFE** **GKKDVAQIFNNILRRQIGTRTPTVEYICTQQNILFMLLKGYESPEIAL** **NCGIMLRECIRHEPLAKIILWSEQFYDFFRYVEMSTFDIASDAFATFK** **DLLTRHKLLSAEFLEQHYDRFFSEYEKLLHSENYVTKRQSLKLLGELL** **LDRHNFTIMTKYISKPENLKLMMNLLRDKSRNIQFEAFHVFKVFVANP** **NKTQPILDILLKNQAKLIEFLSKFQNDRTEDEQFNDEKTYLVKQIRDL** **KRPAQQEA**
Native sequence	Amino acids P2 – A341 (end) of human MO25 alpha. Residue P245 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220 and the MYC tag (*EQKLISEEDL*) is located at residues 235 – 244.
Protease cleavage	Prescission site (LEVLFQGP) at residues 221 – 228
Cloning sites	*Bam*H1 sites of pGex6P-1

Nucleotide sequence of insert

ggatccGCCACCATGGAGCAGAAGCTGATCTCTGAAGAGGACTTGCCG
TTCCCGTTTGGGAAGTCTCACAAATCTCCAGCAGACATTGTGAAGAAT
CTGAAGGAGAGCATGGCTGTTCTGGAAAAGCAAGACATTTCTGATAAA
AAAGCAGAAAAGGCTACAGAAGAAGTTTCCAAAAATCTGGTTGCCATG
AAAGAAATTCTGTATGGCACAAATGAAAAAGAGCCTCAGACAGAAGCA
GTAGCTCAACTTGCTCAAGAACTCTATAATAGTGGGCTCCTTAGCACC
CTGGTAGCTGATTTACAGCTCATTGACTTTGAGGGCAAAAAAGACGTG
GCTCAAATTTTCAACAATATTCTCAGAAGACAAATTGGTACGAGAACT
CCTACTGTTGAATACATCTGCACCCAACAGAATATTTTGTTCATGTTA
TTGAAAGGGTATGAATCTCCAGAAATAGCTCTAAATTGTGGAATAATG
TTAAGAGAATGCATCAGACATGAACCACTTGCAAAAATCATTTTGTGG
TCGGAACAGTTTTATGATTTCTTCAGATATGTCGAAATGTCAACATTT
GACATAGCTTCAGATGCATTTGCCACATTCAAGGATTTACTTACAAGA
CATAAATTGCTCAGTGCAGAATTTTTGGAACAGCATTATGATAGATTT
TTCAGTGAATATGAGAAGTTACTTCATTCAGAAAATTATGTGACAAAA
AGACAGTCACTGAAGCTTCTCGGTGAACTACTACTAGATAGACACAAC
TTCACAATTATGACAAAATACATCAGTAAACCTGAGAACCTCAAATTA
ATGATGAACCTGCTGCGAGACAAAAGTCGCAACATCCAGTTTGAGGCC
TTTCACGTTTTTAAGGTGTTTGTAGCCAATCCTAACAAGACGCAGCCC
ATCCTAGACATCCTCCTCAAGAACCAGGCCAAACTCATAGAGTTCCTC
AGCAAGTTTCAGAACGACAGGACGGAGGATGAGCAGTTTAACGACGAG
AAGACCTATTTAGTTAAACAGATCAGGGATTTGAAGAGACCAGCTCAG
CAAGAAGCTtaaggatcc