

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of M025 alpha [1 - 341] R227A

Enzyme description:- MO25 alpha [1 – 341] R227A

Clone number:- DU 17691

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 66, 565.31 daltons

Average Mass 66, 608.05 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.00

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 [1 - 341] R227A

Protein MO25 alpha [1 - 341] R227A

Clone number DU 17691

Species Human

Accession number NM_016289.3

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMPPFFGKSH
KSPADIVKNLKE SMAVLEKQDISDKKAEKATEEVSKNLVAMKE ILYGT
NEKEPQTEAVAQLAQELYNSGLLSTLVADLQLIDFEGKDV AQIFNNI
LRRQIGTRTPTVEYICTQQNILEMLLKGYESPEIALNCGIMLRECI RH
EPLAKI IILWSEQFYDFFRYVEMSTFDIASDAFATFKDLLTRHKLLSAE
FLEQHYDRFFSEYEKLLHSENYVTKAQSLKLLGELLDRHNFTIMTKY
ISKPENLKLMMNLLRDKSRNIQFEAFHVFKV FVANPNKTQPILDILLK
NQAKLIEFLSKFQNDRTEDEQFNDEKTYLVKQIRDLKRPAQQA

Native sequence Amino acids M1 – A341 (end) of human MO25 alpha.
Residue M232 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
The enzyme has a R227A mutation. Residue R227 is equivalent to A458 of the fusion protein.

Protease cleavage Precission site (LEVLFQGP) at residues 221 – 228

Cloning sites BamH1 sites of pGex6P-1

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Nucleotide
sequence of insert

ggatccATGCCGTTCCCGTTTGGGAAGTCTCACAAATCTCCAGCAGAC
ATTGTGAAGAATCTGAAGGAGAGCATGGCTGTTCTGGAAAAGCAAGAC
ATTTCTGATAAAAAAGCAGAAAAGGCTACAGAAGAAGTTTCCAAAAAT
CTGGTTGCCATGAAAGAAATTCTGTATGGCACAAATGAAAAAGAGCCT
CAGACAGAAGCAGTAGCTCAACTTGCTCAAGAACTCTATAATAGTGGG
CTCCTTAGTACCCTGGTAGCTGATTTACAGCTCATTGACTTTGAGGGC
AAAAAAGACGTGGCTCAAATTTTCAACAATATTCTCAGAAGACAAATT
GGTACGAGAACTCCTACTGTTGAATACATCTGCACCCAACAGAATATT
TTGTTTCATGTTATTGAAAGGGTATGAATCTCCAGAAATAGCTCTAAAT
TGTGGAATAATGTTAAGAGAATGCATCAGACATGAACCACTTGCAAAA
ATCATTTTGTGGTCGGAACAGTTTTATGATTTCTTCAGATATGTGCGAA
ATGTCAACATTTGACATAGCTTCAGATGCATTTGCCACATTCAGGAT
TTACTTACAAGACATAAATTGCTCAGTGCAGAAATTTTTGGAACAGCAT
TATGATAGATTTTTTTCAGTGAATATGAGAAGTTACTTCATTCAGAAAAT
TATGTGACAAAAGCACAGTCACTGAAGCTTCTCGGTGAACTACTACTA
GATAGACACAACCTTCACAATTATGACAAAATACATCAGTAAACCTGAG
AACCTCAAATTAATGATGAACCTGCTGCGAGACAAAAGTCGCAACATC
CAGTTTGAGGCCTTTCACGTTTTTAAAGGTGTTTGTAGCCAATCCTAAC
AAGACGCAGCCCATCCTAGACATCCTCCTCAAGAACCAGGCCAAACTC
ATAGAGTTCCTCAGCAAGTTTTCAGAACGACAGGACGGAGGATGAGCAG
TTTAACGACGAGAAGACCTATTTAGTTAAACAGATCAGGGATTTGAAG
AGACCAGCTCAGCAAGAAGCTtaaggatcc