

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of M025 alpha [1 - 341]**

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

**Clone number:-** DU 2945

**Source:-** Recombinant

**Expression system:-** *E.coli*,

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 66, 650.38 daltons

Average Mass 66, 693.16 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.09

**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]**

|                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Protein</u></b>                       | MO25 alpha [1 - 341]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b><u>Clone number</u></b>                  | DU 2945                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b><u>Species</u></b>                       | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b><u>Accession number</u></b>              | NM_016289.3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b><u>Tags</u></b>                          | N-terminal GST                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b><u>Bacterially expressed protein</u></b> | <p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL<br/>GLEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAIEISMLE<br/>GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN<br/>GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY<br/>LKSSKYIAWPLOGWQATFGGGDHPPKSDLEVLFOGPLGSMPPFGKSH<br/><b>KSPADIVKNLKESSMAVLEKQDISDKKAEKATEEVSKNLVAMKEILYGT</b><br/><b>NEKEPQTEAVAQLAQELYNSGLLSTLVADLQLIDFEGKKDVAQIFNNI</b><br/><b>LRRQIGTRTPTVEYICTQONILFMLLKGYESPEIALNCGIMLRECIRH</b><br/><b>EPLAKIILWSEQFYDFFRYVEMSTFDIASDAFATFKDLLTRHKLLSAE</b><br/><b>FLEQHYDRFFSEYEKLLHSENYVTKRQSLKLLGELLLDRHNFTIMTKY</b><br/><b>ISKPENLKLMMNLLRDKSRNIQFEAFHVFKVFFVANPNKTQPILDILLK</b><br/><b>NQAKLIEFLSKFQNDRTEDeqFNDEKTYLVKQIRDLKRPAQqEA</b></p> |
| <b><u>Native sequence</u></b>               | <p>Amino acids M1 – A341 (end) of human MO25 alpha.<br/>Residue M232 of fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b><u>Protease cleavage</u></b>             | Prescission site ( <u>LEVLFQGP</u> ) at residues 221 – 228                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b><u>Cloning sites</u></b>                 | <i>Bam</i> H1 sites of pGex6P-1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

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Nucleotide  
sequence of insert

ggatccATGCCGTTCCCGTTTGGGAAGTCTCACAAATCTCCAGCAGAC  
ATTGTGAAGAATCTGAAGGAGAGCATGGCTGTTCTGGAAAAGCAAGAC  
ATTTCTGATAAAAAAGCAGAAAAGGCTACAGAAGAAGTTTCCAAAAAT  
CTGGTTGCCATGAAAGAAATTCTGTATGGCACAAATGAAAAAGAGCCT  
CAGACAGAAGCAGTAGCTCAACTTGCTCAAGAACTCTATAATAGTGGG  
CTCCTTAGCACCCCTGGTAGCTGATTTACAGCTCATTGACTTTGAGGGC  
AAAAAAGACGTGGCTCAAATTTTCAACAATATTCTCAGAAGACAAATT  
GGTACGAGAACTCCTACTGTTGAATACATCTGCACCCCAACAGAATATT  
TTGTTTCATGTTATTGAAAGGGTATGAATCTCCAGAAATAGCTCTAAAT  
TGTGGAATAATGTTAAGAGAATGCATCAGACATGAACCACTTGCAAAA  
ATCATTTTGTGGTCGGAACAGTTTTATGATTTCTTCAGATATGTCGAA  
ATGTCAACATTTGACATAGCTTCAGATGCATTTGCCACATTCAAGGAT  
TTACTTACAAGACATAAATTGCTCAGTGCAGAAATTTTGGAACAGCAT  
TATGATAGATTTTTCAGTGAATATGAGAAGTTACTTCATTCAGAAAAT  
TATGTGACAAAAAGACAGTCACTGAAGCTTCTCGGTGAACTACTACTA  
GATAGACACAACCTTCACAATTATGACAAAATACATCAGTAAACCTGAG  
AACCTCAAATTAATGATGAACCTGCTGCGAGACAAAAGTCGCAACATC  
CAGTTTGAGGCCTTTCACGTTTTTAAGGTGTTTGTAGCCAATCCTAAC  
AAGACGCAGCCCATCCTAGACATCCTCCTCAAGAACCAGGCCAACTC  
ATAGAGTTCCTCAGCAAGTTTCAGAACGACAGGACGGAGGATGAGCAG  
TTTAACGACGAGAAGACCTATTTAGTTAAACAGATCAGGGATTTGAAG  
AGACCAGCTCAGCAAGAAGCTtaaggatcc