

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of MO25 beta [5 - 337]**

**Enzyme description:-** MO25 beta [5 – 337]

**Clone number:-** DU 38053

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 65, 595.26 daltons

Average Mass 65, 637.32daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.43

**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 beta [5 - 337]**

<b><u>Protein</u></b>	MO25 beta [5 - 337]
<b><u>Clone number</u></b>	DU 38053
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_030925.2
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSML<b>PLFSKSH</b> <b>KNPAEIVKILKDNLAILEKQDKKTDKASEEVSKSLQAMKEILCGTNEK</b> <b>EPPTEAVAQLAQELYSSGLLVTLIADLQLIDFEGKKDVTQIFNNILRR</b> <b>QIGTRSPTVEYISAHPHILFMLLKGYEAPQIALRCGIMLRECI RHEPL</b> <b>AKIILFSNQFRDFFKYVELSTFDIASDAFATFKDLLTRHKVLVADFLE</b> <b>QNYDTIFEDYEKLLQSENYVTKRQSLKLLGELILDRHNF AIMTKYISK</b> <b>PENLKLMMNLLRDKSPNIQFEAFHVFKV FVASPHKTQPIVEILLKNQP</b> <b>KLIEFLSSFQKERTDDEQFADEKNYLIKQIRDLKKTAP</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids P5 – P337 (end) of human MO25 beta. Residue P234 of fusion protein is equivalent to P5 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<b><u>Protease cleavage</u></b>	Prescission site ( <u>LEVLFOGP</u> ) at residues 221 – 228
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGex6P-1

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**Nucleotide  
sequence of insert**

ggatccatggttgCCTTTGTTTAGTAAATCACACAAAAATCCAGCAGAA  
ATTGTGAAAATCCTGAAAGACAATTTGGCCATTTTGGAAAAGCAAGAC  
AAAAAGACAGACAAGGCTTCAGAAGAAGTGTCTAAATCACTGCAAGCA  
ATGAAAGAAATTCTGTGTGGTACAAACGAGAAAGAACCCCGACAGAA  
GCAGTGGCTCAGCTAGCACAAAGAACTCTACAGCAGTGGCCTGCTGGTG  
ACACTGATAGCTGACCTGCAGCTGATAGACTTTGAGGGAAAAAAGAT  
GTGACCCAGATATTTAACAACATCTTGAGAAGACAGATAGGCACTCGG  
AGTCTACTGTGGAGTATATTAGTGCTCATCCTCATATCCTGTTTATG  
CTCCTCAAAGGATATGAAGCCCCACAGATTGCCTTACGTTGTGGGATT  
ATGCTGAGAGAATGTATTCGACATGAACCACTTGCCAAAATCATCCTC  
TTTTCTAATCAATTCAGAGATTTCTTTAAGTACGTGGAGTTGTCAACA  
TTTGATATTGCTTCAGATGCCTTTGCTACTTTCAAGGATTTACTAACC  
AGACATAAAGTGTTGGTAGCAGACTTCTTAGAACAAAATTACGACACT  
ATTTTTGAAGACTATGAGAAATTGCTTCAGTCTGAGAATTATGTTACT  
AAGAGACAGTCTTTAAAGCTGCTAGGGGAGCTGATCCTGGACCGTCAC  
AACTTTGCCATCATGACAAAGTATATCAGCAAGCCGGAGAACCTGAAA  
CTCATGATGAACCTCCTTCGGGATAAAAAGTCCCAACATCCAGTTTGAA  
GCCTTTCATGTTTTTAAGGTGTTTGTGGCCAGTCCTCACAAAACACAG  
CCTATTGTGGAGATCCTGTAAAAAATCAGCCAAACTCATTGAGTTT  
CTGAGCAGTTCCAAAAAGAAAGACGGATGATGAGCAGTTCGCTGAC  
GAGAAGAACTACTTGATTAAACAGATCCGAGACTTGAAGAAAACGGCC  
CCTtgagcggccgc