

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of MYO3B [1 - 326]

Enzyme description:- MYO3B [1 - 326]

Clone number:- DU 68068

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 63, 783.59 daltons

Average Mass 63, 824.87 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.17

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay Buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-

Myelin Basic protein Final concentration: 1 mg/ml

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Clone Data Sheet

MYO3B [1 - 326]

<u>Protein</u>	MYO3B [1 - 326]
<u>Clone number</u>	DU 68068
<u>Species</u>	Human
<u>Accession number</u>	NM_138995.4
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMKHLYGLFHYNPMM LGLES LPDPTDWEI IETIGKGTYGKVYKVTNKR DGS LAAVKILDPVSD MDEEIEAEYNILQFLPNHPNVVKFYGMFYKADHCVGGQLWLVL ELCNGG SVTELVKGLLR CGQRLDEAMISYILYGALLGLQH LHNRI IHRDVKGNN ILLTTEGGVKLVDFGVSAQLTSTR LRRNTSVGTPFWMAPEVIACEQQYD SSYDARCDVWSLGITAIELGDGDPPLFDMHPVKTLFKIPRNPPPTLLHP EKWCEEFNHFISQCLIKDFERRPSVTHLLDHPFIKGVH GKVLFLQQLA KVLQDQKHQNPVAKTRHE
<u>Native sequence</u>	Amino acids M1 – E326 (end residue is H1341) of human MYO3B. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (LEVLFQGP) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pGEX6P-1

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Nucleotide Sequence Of Insert

ggatccATGAAACATCTGTATGGATTATTTCACTATAATCCTATGATGC
TTGGACTTGAATCACTTCCAGATCCCACAGACACCTGGGAAATTATAGA
GACCATTGGTAAAGGCACCTATGGCAAAGTCTACAAGGTAAC TAACAAG
AGAGATGGGAGCCTGGCTGCAGTGAAAATTCTCGATCCAGTCAGTGATA
TGGATGAAGAAATTGAGGCAGAATACAACATTTTGCAGTTCCTTCCTAA
TCATCCCAATGTTGTAAAGTTTTATGGGATGTTTTACAAAGCGGATCAC
TGTGTAGGGGGACAGCTGTGGCTGGTCCTGGAGCTGTGTAATGGGGGCT
CAGTCACTGAGCTTGTCAAAGGTCTACTCAGATGTGGCCAGCGGTTGGA
TGAAGCAATGATCTCATA CATCTTGTACGGGGCCCTCTTGGGCCCTCAG
CATTTGCACAACAACCGAATCATCCACCGTGATGTGAAGGGGAATAACA
TTCTTCTGACAACAGAAGGAGGAGTTAAGCTCGTTGACTTTGGTGTTTC
AGCTCAACTCACCAGTACACGTCTGCGGAGAAACACATCTGTTGGCACC
CCGTTCTGGATGGCCCCCTGAGGTCATTGCCTGTGAGCAGCAGTATGACT
CTTCCTATGACGCTCGCTGTGACGTCTGGTCCTTGGGGATCACAGCTAT
TGA ACTGGGGGATGGAGACCCTCCCCTCTTTGACATGCATCCTGTGAAA
ACACTCTTTAAGATTCCAAGAAATCCTCCACCTACTTTACTTCATCCAG
AAAAATGGTGTGAAGAATTCAACCACTTTATTTACAGTGTCTTATTAA
GGATTTTGAAGGCGACCTTCCGTCACACATCTCCTTGACCACCCATTT
ATTAAAGGAGTACATGGAAAAGTTCTGTTTCTGCAAAAACAGCTGGCCA
AGGTTCTCCAAGACCAGAAGCATCAAAATCCTGTTGCTAAAACCAGGCA
TGAGtgagcggccgc