

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of PYGM [1 – 842]

Enzyme description:- PYGM [1 - 842]

Clone number:- DU 8487

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 125, 009.88 daltons

Average Mass 125, 089.57 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.44

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

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Clone Data Sheet

PYGM [1 - 842]

<u>Protein</u>	PYGM [1 - 842]
<u>Clone number</u>	DU 8487
<u>Species</u>	Mouse
<u>Accession number</u>	AAD30476.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFGQPLGSPGIPGSTRAAAMSR PLSDQDKRKQISVRGLAGVENVSELKKNFNRLHFTLVKDRNVATPRDY YFALAHTVRDHLVGRWIRTQOHYIEKDPKRIYYLSLEFYMGRTLQNTMV NLALENACDEATYQLGLDMEELIEEDAGLGNGGLGRLAACFLDSMAT LGLAAYGYGIRYEFGIFNQKICGGWQMEEADDWLRYPWEKARPEFTL PVHFYGRVEHTSQGAKWVDTQVVLAMPYDTPVPGYRNNVVNTMRLWSAK APNDFNLKDFNVGGYIQAVLDRNLAENISRVLYPNDNFFEGKELRLKQE YFVVAATLQDIIRRFKSSKFGSRDPVRTNFDAFPDKVAIQLNDRHPSLA IPELMRILVDLERLDWDKAWDVTVKTCAYTNHTVLPALERWPVHLMET LLPRHLQIIEINQRFLNRVAAAFPGDVDRLRRMSLVEEGAVKRINMAH LCIAGSHAVNGVARIHSEILKKTIFKDFYELEPHKFQNKTNGITPRRWL VLCNPGLAEVIAERIGEDIISDLQLRKLLSYVDDEAFIRDVAKVKQEN KLKFSAYLEREYKVHINPNSLFDVQVKRIHEYKRQLLNCLHIITLYNRI KREPNRFMVPRTIMIGGKAAPGYHMAKMIKILITAIIGDVVNHDPAVGDR LRVIFLENYRVSLAEKVI PAADLSEQISTAGTEASGTGNMKFMLNGALT IGTMDGANVEMAE EAGEENFFIFGMRVEDVERLDQRGYNAQEYYDRIP LRQIEQLSSGFFSPKQPDFKDIVNMLMHDRFKVFADYEEYIKCQDK VSELYKNPREWTRMVIRNIATSGKFS SDRITIAQYAREIWGVPEPSRQRLP APDEKI</p>
<u>Native sequence</u>	<p>Amino acids M1 – I842 (end) of mouse PYGM. Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (LEVLFGQP) residues 221 – 228
<u>Cloning sites</u>	<i>Not1</i> sites of pGEX6P-1

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Nucleotide Sequence of insert

gcggccgcgATGTCCAGGCCTCTTTTCAGACCAGGATAAGAGAAAGCAAAT
CAGCGTTCGTGGCTTAGCCGGAGTGGAAAATGTGTCTGGAGCTAAAAAGA
ACTTCAACCGCCACCTGCATTTACCTTGGTCAAGGATCGCAATGTGGCT
ACTCCGAGAGATTACTATTTTGCCTGGCCACACTGTCCGGGACCACCT
CGTGGGACGCTGGATCCGCACACAGCAGCATTACTACGAAAAGGACCCCA
AGAGGATCTACTACCTGTCTTTGGAGTTCTACATGGGCCGGACACTGCAG
AACACCATGGTGAACCTGGCCTTGGAGAACGCCTGTGATGAGGCCACTTA
CCAGCTGGGCTTGGACATGGAGGAGTTGGAGGAGATCGAGGAGGATGCAG
GGTTGGGCAATGGGGCCTGGGACGCCTGGCAGCTTGCTTTTGGACTCC
ATGGCTACCCTGGGGCTCGCTGCCTATGGCTATGGGATCCGCTATGAGTT
TGGGATTTTTAATCAGAAGATCTGTGGGGGCTGGCAGATGGAAGAAGCTG
ATGACTGGCTTCGCTATGGCAACCCCTGGGAGAAGGCCCGCCAGAGTTC
ACGCTGCCTGTGCATTTCTATGGCCGAGTGGAGCACACCAGCCAGGGTGC
CAAGTGGGTGGACACACAGGTAGTGCTGGCTATGCCCTATGACACTCCTG
TGCCCGGCTATCGGAACAACGTCGTCAACACCATGCGCCTCTGGTCGGCC
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AGCGGCTTCTTCTCCCCAAGCAGCCTGACCTGTTCAAGGACATTGTCAA
TATGCTCATGCACCATGACCGGTTTAAAGTCTTTGCAGATTATGAGGAAT
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TGGACACGGATGGTGTGATCAGGAACATAGCCACCTCTGGCAAGTTTTCCAG
TGACCGCACCATTGCCCAGTATGCCCGGAGATCTGGGGTGTGAAACCTT
CTCGCCAGCGCTGCCAGCCCCAGATGAGAAGATCtgagcggccgc

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