

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of PYGL [1 – 847]**

**Enzyme description:-** PYGL [1 - 847]

**Clone number:-** DU 9035

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic 124, 474.03 daltons

Average Mass 124, 553.49 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.46

**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**

**PYGL [1 - 847]**

Protein PYGL [1 - 847]

Clone number DU 9035

Species Human

Accession number NM\_002863.4

Tags N-terminal GST

Bacterially  
expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPLPYYIDGDVKLQSMAIIRYIADKHNLGGCPKERAEISMLEGAV  
LDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHV  
THPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSK  
YIAWPLQGWQATFGGGDHPKSDLEVL FQGPLGSGIRPLGT**MAKPLTDQ**  
**EKRRQISIRGIVGVENVAELKKSFNRLHFTLVKDRNVATTRDYFALA**  
**HTVRDHLVGRWIRTQOHYDKCPKRVYYSLEFYMGRTLQNTMINLGLQ**  
**NACDEAIYQLGLDIEELEIEEDAGLGNGLGRLAACFLDSMATLGLAA**  
**YGYGIRYEYGIFNQKIRDGWQVEEADDWLRYGNPWEKS RPEFMLPVHFY**  
**GKVEHTNTGTKWIDTQVVLALPYDTPVPGYMNNTVNTMRLWSARAPNDF**  
**NLRDFNVGDYIQAVLDRNLAENISRVLYPNDNFFEGKELRLKQ EYFVVA**  
**ATLQDIIRRFKASKFGSTRGAGTVFDAFPDQVAIQLN DTHPALAIPELM**  
**RIFVDIEKLPWSKAWELTQKTFAYTNHTVLPEALERWPVDLVEKLLPRH**  
**LEI IYEINQKHLDRIVALFPKDVDR LRRMSLIEEEGSKRINMAHLCIVG**  
**SHAVNGVAKIHSDIVKTKVFKDFSELEPKDFQNK TNGITPRRWLLCNP**  
**GLAELIAEKIGEDYVKDLSQLTKLHSFLGDDVFLRELAKVKQENKLF S**  
**QFLETEYKVKINPSSMFDVQVKRIHEYKRQLLNCLHVI TMYNRIKKDPK**  
**KLFVPRTVIIGGKAAPGYHMAKMI IKLITSVADVNN DPMVGSKLKVIF**  
**LENYRVSLAEKVI PATDLSEQI STAGTEASGTGNMKFMLNGALTIGTMD**  
**GANVEMAE EAGEENLFI FGMRIDDVAALDKKGYEAKEYYEALPELKLVI**  
**DQIDNGFFSPKQPD LFKDI INMLFYHDRFKVFADYEAYVKCQDKVSQLY**  
**MNPKAWNTMVLKNIAASGKFS SDRTIKEYAQNIWNVEPSDLKISLSNES**  
**NKVNGN**

Native sequence Amino acids M1 – N847 (end) of human PYGL.  
Residue M238 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVL FQGP) residues 221 – 228

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### Cloning sites

*Bam*H1 sites of pGEX6P-1

### Nucleotide Sequence of insert

ggatccggaattcgccctttaggtaccATGGCGAAACCCCTGACGGACCAGGAGAAGCGGCGGCAG  
ATCAGCATCCGCGGCATCGTGGGCGTGGAGAACGTGGCAGAGCTGAAGAAGAGTTTCAACCGGCAC  
CTGCACTTCACGCTGGTCAAGGACCACAACGTGGCCACCACCCGCGACTACTACTTCGCGCTGGCG  
CACACGGTGCAGCACCACCTGGTGGGGCGCTGGATCCGCACGCAGCAGCACTACTACGACAAGTGC  
CCCAAGAGGGTATATTACCTCTCTCTGGAATTTTACATGGGCCGAACATTACAGAACACCATGATC  
AACCTCGGTCTGCAAAATGCCTGTGATGAGGCCATTTACCAGCTTGGATTGGATATAGAAGAGTTA  
GAAGAAATTGAAGAAGATGCTGGACTTGGCAATGGTGGTCTTGGGAGACTTGCTGCCTGCTTCTTG  
GATTCATGGCAACCCTGGGACTTGCAGCCTATGGATACGGCATTTCGGTATGAATATGGGATTTTC  
AATCAGAAGATCCGAGATGGATGGCAGGTAGAAGAAGCAGATGATTGGCTCAGATATGGAAACCT  
TGGGAGAAGTCCCGCCAGAATTCATGCTGCCGTGTGCACTTCTATGGAAAAGTAGAACACACCAAC  
ACGGGGACCAAGTGGATTGACACTCAAGTGGTCTGGCTCTGCCATATGACACCCCGTGCCTGGC  
TACATGAATAACACTGTCAACACCATGCGCCTCTGGTCTGCTCGGGCACCAAATGACTTTAACCTC  
AGAGACTTTAATGTTGGAGACTACATTCAGGCTGTGCTGGACCGAAACCTGGCCGAGAACATCTCC  
CGGGTCTCTATCCCAATGACAATTTTTTTGAAGGGAAGGAGCTAAGATTGAAGCAGGAATACTTT  
GTGGTGGCTGCAACCTTGCAAGATATCATCCGCCGTTTCAAAGCCTCCAAGTTTGGCTCCACCCGT  
GGTGCAGGAACCTGTGTTGATGCCTTCCCGGATCAGGTGGCCATCCAGCTGAATGACACTCACCT  
GCACTCGCGATCCCTGAGCTGATGAGGATTTTTGTGGATATTGAAAACTGCCCTGGTCCAAGGCA  
TGGGAGCTCACCCAGAAGACCTTCGCCTACACCAACCACACAGTGTCTCCCGGAAGCCCTGGAGCGC  
TGGCCCGTGGACCTGGTGGAGAAGCTGCTCCCTCGACATTTGGAATCATTATGAGATAAATCAG  
AAGCATTTAGATAGAATTGTGGCCTTGTTCCTAAAGATGTGGACCGTCTGAGAAGGATGTCTCTG  
ATAGAAGAGGAAGGAAGCAAAAGGATCAACATGGCCCATCTCTGCATTGTCTGGTTCCCATGCTGTG  
AATGGCGTGGCTAAAATCCACTCAGACATCGTGAAGACTAAAGTATTCAAGGACTTCAGTGAGCTA  
GAACCTGACAAGTTTCAGAATAAAACCAATGGGATCACTCCAAGGCGCTGGCTCCTACTCTGCAAC  
CCAGGACTTGCAGAGCTCATAGCAGAGAAAATTTGGAGAAGACTATGTGAAAGACCTGAGCCAGCTG  
ACGAAGCTCCACAGCTTCTGGGTGATGATGTCTTCTCCGGGAACCTCGCCAAGGTGAAGCAGGAG  
AATAAGCTGAAGTTTTCTCAGTTCCTGGAGACGGAGTACAAAGTGAAGATCAACCCATCCTCCATG  
TTTGATGTCCAGGTGAAGAGGATACATGAGTACAAGCGACAGCTCTTGAAGTGTCTGCATGTGATC  
ACGATGTACAACCGCATTAAGAAAGACCCTAAGAAGTTATTTCGTGCCAAGGACAGTTATCATTTGGT  
GGTAAAGCTGCCCCAGGATATCACATGGCCAAAATGATCATAAAGCTGATCACTTCAGTGGCAGAT  
GTGGTGAACAATGACCCATGGTTGGAAGCAAGTTGAAAGTCATCTTCTTGGAGAACTACAGAGTA  
TCTCTTGCTGAAAAAGTCATTCCAGCCACAGATCTGTGAGAGCAGATTTCCACTGCAGGCACCGAA  
GCCTCGGGGACAGGCAATATGAAGTTCATGCTAAATGGGGCCCTAACTATCGGGACCATGGATGGG  
GCCAATGTGGAATGGCAGAAGAAGCTGGGGAAGAGAACCTGTTTTCATCTTTGGCATGAGGATAGAT  
GATGTGGCTGCTTTGGACAAGAAAGGGTACGAGGCAAAAGAATACTATGAGGCACTTCCAGAGCTG  
AAGCTGGTCAATTGATCAAATTGACAATGGCTTTTTTTCTCCCAAGCAGCCTGACCTCTTCAAAGAT  
ATCATCAACATGCTATTTTATCATGACAGGTTTAAAGTCTTTGCGAGACTACGAAGCCTATGTCAAG  
TGTCAGATAAAGTGAGTCAGCTGTACATGAATCCAAAGGCTGGAACACAATGGTACTCAAAAAC  
ATAGCTGCCTCGGGGAAATTTCTCCAGTGACCGAACCAATTAAGAATATGCCCAAAACATCTGGAAC  
GTGGAACCTTCAGATCTAAAGATTTCTCTATCCAATGAATCTAACAAAGTCAACGGTAATTAGAAG  
GGCAATTCAGCACACTGGCGGCCGTTACTagtggtaccatcgatgcgggccg

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