

## ***MRC PPU REAGENTS***

### **Standard Operating Procedure**

#### **Preparation of PGAM5 [1 – 289]**

<b><u>Enzyme description:-</u></b>	PGAM5 [1 - 289]
<b><u>Clone number:-</u></b>	DU 63508
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	<i>E.coli</i>
<b><u>Tag:-</u></b>	N-terminal GST and C-terminal HA
<b><u>Purification method:-</u></b>	GSH Sepharose

#### **Calculated molecular mass:-**

Monoisotopic            59, 874.70 daltons  
Average Mass            59, 912.77 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                                    6.62

#### **Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 270mM 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

### PGAM5 [1 - 289]

<u>Protein</u>	PGAM5 [1 - 289]
<u>Clone number</u>	DU 61135
<u>Species</u>	Human
<u>Accession number</u>	NM_001170543.2
<u>Tags</u>	N-terminal GST and C-terminal HA
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQG PLGSMAFRQALQLAACGL <b>AGGSAAVLFS AVAVGKPRAGGDAEPRPAEPPAWAGGARPGPGVWDPNWD</b> <b>RREPLSLINVRKRNVESGEEELASKLDHYKAKATRHI FLIRHSQYHVDG</b> <b>SLEKDRTLTP LGREQAELTGLRLASLGLKFNKIVHSSMTRAIETTDIIS</b> <b>RHLPGVCKVSTDLLREGAPIEPDPPVSHWKPEAVQYYEDGARIEAAFRN</b> <b>YIHRADARQEEDSYEIFICHANVIRYIVCRALQFPPEGWLRSLNNGSI</b> <b>THLVIRPNGRVALRTLGD TGFMPPDKITRSYPYDVPDYA</b></p>
<u>Native sequence</u>	<p>Amino acids M1 – S289 (end) of human PGAM5. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220 and the HA [YPYDVPDYA] tag is located at residues 521 - 529.</p>
<u>Protease cleavage</u>	PreScission (LEVL FQGP) residues 221 – 228.
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGex6P1

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### Nucleotide Sequence Of Insert

ggatccATGGCGTTCCGGCAGGCGCTGCAGCTGGCGGCCTGCGGGCTGGCCGGGGGCTCGG  
CCGCCGTGCTCTTCTCGGCCGTGGCGGTAGGGAAGCCGCGCGCAGGCCGGGACGCGGAGCC  
ACGCCCCGGCTGAGCCGCCGGCCTGGGCGGGGGCGCGCGGCCGGGCCCCGGTGTCTGGGAC  
CCCAACTGGGACAGGCGAGAACCCTGTCTCTGATCAACGTGCGGAAGAGGAACGTGGAAT  
CTGGGGAAGAAGAGCTGGCGTCCAAGCTGGACCACTACAAAGCCAAGGCCACGCGGCACAT  
CTTCCTCATCAGGCATTCCAGTACCACGTGGATGGCTCCCTGGAGAAGGACCGCACTCTG  
ACCCCGCTGGGTGGGAGCAGGCTGAACTCACTGGGCTCCGCCTGGCAAGCTTGGGGTTGA  
AGTTTAATAAAAATCGTCCATTTCGTCTATGACGCGCGCCATAGAGACCACCGATATCATCAG  
CCGGCACCTGCCAGGCGTCTGCAAAGTCAGCACAGATCTGCTGCGGGAAGGCGCCCCATC  
GAGCCAGACCCGCCCGTGTCTCATTGGAAGCCGGAAGCTGTGCAGTATTACGAAGACGGAG  
CCCGGATCGAGGCCGCCCTTCCGGAACTACATCCACCGCGCAGATGCCAGGCAGGAGGAGGA  
CAGTTACGAGATCTTCATCTGTCACGCCAACGTCATCCGCTACATCGTGTGCAGAGCACTG  
CAGTTTCCTCCTGAAGGCTGGCTCCGGCTCTCCCTCAATAATGGCAGCATCACCCACCTGG  
TGATCCGACCCAACGGCCGAGTTGCGCTCAGGACCCTCGGGGACACGGGGTTCATGCCTCC  
CGACAAGATCACTCGATCCTACCCATACGATGTGCCAGATTACGCCt g a g c g g c c g c