

# University of Dundee

## Standard Operating Procedure

### Preparation of active Phosphorylase kinase gamma subunit [2 – 297] (muscle isoform)

**Enzyme description:-** Phosphorylase kinase gamma subunit [2 – 297]  
(muscle isoform)

**Clone number:-** DU 4030

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Expression level:-** 6 mg/L

**Calculated molecular mass:-** 60, 513 daltons

**Purity:-** >80 %

**Activation protocol:-** Constitutively active

**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, 0.2 mM PMSF, 1 mM Benzamidine

**Storage temperature:-** -70 °C

**Assay:-** Standard filter binding assay

**Assay buffer:-**

50 mM Tris-HCl pH 8.6, 50 mM Na-2-glycerolphosphate, 40  $\mu$ M CaCl<sub>2</sub>, 10 mM MgAc

**Substrate:-**

KRKQISVRGL      Residues 10 – 19 of phosphorylase b  
Final concentration: 300  $\mu$ M

**Specific activity range:-** 100 – 200 U/mg

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## Clone Data Sheet - Phosphorylase kinase gamma subunit [2 - 297] (muscle isoform)

**Protein** Phosphorylase Kinase gamma subunit [2 - 297] (muscle isoform)

**Clone number** DU 4030

**Species** Human

**Accession number** X80590

**Tags** N-terminal GST

**Bacterially expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKLTQSMALIRYIADKHNMLGGCPKERAELSMLE  
GAVLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLQGWQATFGGGDHPKSDLVPRGSTRDEALPDSHSAQD  
FYENYEPKEILGRGVSSVVRRCIHKPTSQEYAVKVIDVTGGGSFSPEE  
VRELREATLKEVDILRKVSGHPNIIQLKDTYETNTFFFLVFDLMKRGE  
LFDYLTEKVTLSEKETRKIMRALLEVIC TLHKLNI VHRDLKPENILLD  
DNMNIKLTDFGFSCQLEPGERLREVC GTPSYLAPEI IEC SMNEDHPGY  
GKEVDMWSTGVIMYTL LAGSPPFWHRKQMLMRMIMSGNYQFGSPEWD  
DYSDTVKDLVSRFLV VQPQNRYTAEALAHPPFFQOYLVEEVR

**Native sequence** Amino acids T2 – R297 of human muscle Phosphorylase kinase gamma subunit. [Full length protein ends at residue Y387]  
Residue T227 of the fusion protein is equivalent to T2 of the native enzyme. The GST tag is located at residues 1 - 220.

**Protease cleavage** Thrombin (LVPRGS) residues 221 - 226

**Cloning sites** *Bam*H1 and *Eco*R1 of pGEX 4T

**Nucleotide sequence**

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCC  
ACTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTG  
TATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTG  
GGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAA  
TTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC  
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAA  
GGAGCGGTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGT  
AAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAA  
ATGCTGAAAATGTTCAAGATCGTTTATGTCATAAAACATATTTAAAT  
GGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT

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GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAATTA  
GTTTGTTTTAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTAC  
TTGAAATCCAGCAAGTATATAGCATGGCCTTTCAGAGGCTGGCAAGCC  
ACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGTTCCGCGT  
GGATCC**ACCCGGGACGAGGCACTGCCGGACTCTCATTCTGCACAGGAC**  
**TTCTATGAGAATTATGAGCCAAAGAGATCCTGGGCAGGGGCGTTAGC**  
**AGTGTGGTCAGGCGATGCATCCACAAGCCACGAGCCAGGAGTACGCC**  
**GTGAAGGTCATCGACGTCACCGGTGGAGGCAGCTTCAGCCCGGAGGAG**  
**GTGCGGGAGCTGCGAGAAGCCACGCTGAAGGAGGTGGACATCCTGCGC**  
**AAGTCTCAGGGCACCCAAACATCATAACAGCTGAAGGACACTTATGAG**  
**ACCAACACTTTCTTCTTCTTGGTGTTTGACCTGATGAAGAGAGGGGAG**  
**CTCTTTGACTACCTCACTGAGAAGGTCACCTTGAGTGAGAAGGAAACC**  
**AGAAAGATCATGCGAGCTCTGCTGGAGGTGATCTGCACCTTGCACAAA**  
**CTCAACATCGTGCACCGGGACCTGAAGCCCGAGAACATTCTCTGGAT**  
**GACAACATGAACATCAAGCTCACAGACTTTGGCTTTTCTGCCAGCTG**  
**GAGCCGGGAGAGAGGCTGCGAGAGGTCTGCGGGACCCCAAGTTACCTG**  
**GCCCCTGAGATTATCGAGTGCTCCATGAATGAGGACCACCCGGGCTAC**  
**GGGAAAGAGGTGGACATGTGGAGCACTGGCGTCATCATGTACACGCTG**  
**CTGGCCGGCTCCCCGCCCTTCTGGCACCGGAAGCAGATGCTGATGCTG**  
**AGGATGATCATGAGCGGCAACTACCAGTTTGGCTCGCCCGAGTGGGAT**  
**GATTACTCGGACACCGTGAAGGACCTGGTCTCCCGATTCTGGTGGTG**  
**CAACCCAGAACCGCTACACAGCGGAAGAGGCCTTGGCACACCCCTTC**  
**TTCCAGCAGTACTTGGTGGAGGAAGTGCGGTAAGAATTC**