

## *Division of Signal Transduction Therapy*

## Standard Operating Procedure

## Preparation of active PKC gamma [1 - 697]

|                                     |                                      |
|-------------------------------------|--------------------------------------|
| <b><u>Enzyme description:-</u></b>  | PKC gamma [1 – 697]                  |
| <b><u>Clone number:-</u></b>        | DU 30188                             |
| <b><u>Source:-</u></b>              | Recombinant                          |
| <b><u>Expression system:-</u></b>   | Baculovirus expression vector system |
| <b><u>Tag:-</u></b>                 | N-terminal GST                       |
| <b><u>Purification method:-</u></b> | GSH Sepharose                        |

**Calculated molecular mass:-**

|                                                         |                    |
|---------------------------------------------------------|--------------------|
| Monoisotopic                                            | 105,203.80 daltons |
| Average Mass                                            | 105,272.00 daltons |
| [cysteines reduced, methionines have not been oxidised] |                    |

**Theoretical pI:-** 6.65

**Purity:-** >80 %

|                              |                       |
|------------------------------|-----------------------|
| <b>Activation protocol:-</b> | Constitutively active |
|------------------------------|-----------------------|

**Enzyme storage buffer:-**

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

**Storage temperature:-** -70 °C

### **Assay buffer:-**

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc, 0.1 mM CaCl<sub>2</sub>, 0.1 mg/ml PtdSerine, 10 ug/ml DAG

**Substrate:-**

|            |                                |
|------------|--------------------------------|
| Histone H1 | Final concentration: 0.1 mg/ml |
|------------|--------------------------------|

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### **Clone Data Sheet**

#### **PKC gamma [1 - 697]**

**Protein** PKC gamma [1 - 697]

**Clone number** DU 30188

**Species** Human

**Accession number** NM\_002739

**Tags** N-terminal GST

**Bacterially  
expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL  
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAIEISMLE  
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN  
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY  
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMAGLGPGVG  
**DSEGGPRPLFCRKALRQKVVEVKSHKFTARFFKQPTFCSHCTDFIW**  
**GIGKQGLQCQVCSFVVHRRCHEFVTFECPGAGKGPQTDDPRNKHKFR**  
**HSYSSPTFCDHCGSLLYGLVHQGMKSCCEMNVHRCVRSVPSLCGVD**  
**HTERRGRLQLEIRAPTADEIHVTVGEARNLIPMDPNGLSDPYVKLKL**  
**IPDPRNLTKQKTRTVKATLNPVWNETFVFNLPKPGDVERRLSVEVWDWR**  
**TSRNDFMGAMSFVSELLKAPVDGWYKLLNQEEGEYYNVPVADADNCS**  
**LLQKFEACNYPLELYERVRMGPPSSSPIPSPSPSPPTDPKRCFFGAS**  
**PGRLHISDFSFLMVLGKGSFGKVMLAERRGSDELYAIKILKKDVI**  
**VQDDVDCTLVEKRVLALGGRGPGGRPHFLTQLHSTFQTPDRLYFVMEY**  
**VTGGDLMYHIQQLGKFKEPHAAFYAAEIAIGLFFLHNQGIYRDLKLD**  
**NVMLDAEGHIKITDFGMCKENVFPGTTTTRTFCTGTPDYIAPEIIAYQ**  
**PYGKSVDWWSFGVLLYEMLAGQPPFDGEDEEELFQAIMEQTVTPKSL**  
**SREAVAI CKGFLTKHPGKRLGSGPDGEPTIRAHGFFRWIDWERLERLE**  
**I PPPFRP RPCGRSGENFDKFFTRAAPALTPPDRLVLASIDQADFQGF**  
**TYVNPDFV HPDARSPTSPVPVPM**

**Native sequence** Amino acids M1 – M697 of human PKC gamma.

Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

**Protease cleavage** PreScission site (LEVLFQGP) residues 221 – 228

**Cloning sites** *Bam*H1 and *Not*I sites of pFB GST 6P

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### Nucleotide sequence of insert

ggatccATGGCTGGTCTGGGCCCCGGCGTAGGCGATTcAGAGGGGGGA  
CCCCGGCCCCTGTTTTGCAGAAAGGGGGCCCTGAGGCAGAAGGTGGTC  
CACGAAGTCAAGAGCCACAAGTTCACCGCTCGCTTCTTCAAGCAGCCC  
ACCTTCTGCAGCCACTGCACCGACTTCATCTGGGGTATCGGAAAGCAG  
GGCCTGCAATGTCAAGTCTGCAGCTTTGTGGTTCATCGACGATGCCAC  
GAATTTGTGACCTTCGAGTGTCCAGGCGCTGGGAAGGGCCCCCAGACG  
GATGACCCCCGGAACAAACACAAGTTCCGCCTGCATAGCTACAGCAGC  
CCCACCTTCTGCGACCACTGTGGCTCCCTCCTCTACGGGCTTGTGCAC  
CAGGGCATGAAATGCTCCTGCTGCGAGATGAACGTGCACCGGCGCTGT  
GTGCGTAGCGTGCCCTCCCTGTGCGGTGTGGACCACACCGAGCGCCGC  
GGGCGCCTGCAGCTGGAGATCCGGGCTCCCACAGCAGATGAGATCCAC  
GTAAGTGTGGCGAGGCGCGTAACCTAATTCCTATGGACCCCAATGGT  
CTCTCTGATCCCTATGTGAACTGAAGCTCATCCCAGACCCCTCGGAAC  
CTGACGAAACAGAAGACCCGAACGGTGAAAGCCACGCTAAACCTGTG  
TGGAATGAGACCTTTGTGTTCAACCTGAAGCCAGGGGATGTGGAGCGC  
CGGCTCAGCGTGGAGGTGTGGGACTGGGACCGGACCTCCCGCAACGAC  
TTCATGGGGGCCATGTCTTTGGCGTCTCGGAGCTGCTCAAGGCGCCC  
GTGGATGGCTGGTACAAGTTACTGAACCAGGAGGAGGGCGAGTATTAC  
AATGTGCCGGTGGCCGATGCTGACAACCTGCAGCCTCCTCCAGAAGTTT  
GAGGCTTGTAACACCCCTGGAATTGTATGAGCGGGTGCGGATGGGC  
CCCTCTTCCTCTCCATCCCTCCCTTCCCTAGTCCCACCGACCCC  
AAGCGCTGCTTCTTCGGGGCGAGTCCAGGACGCCTGCACATCTCCGAC  
TTCAGCTTCCTCATGGTTCTAGGAAAAGGCAGTTTTTGGGAAGGTGATG  
CTGGCCGAGCGCAGGGGCTCTGATGAGCTCTACGCCATCAAGATCTTG  
AAAAAGGACGTGATCGTCCAGGACGACGATGTGGACTGCACGCTGGTG  
GAGAAACGTGTGCTGGCGCTGGGGGGCCGGGGTCTGGCGGCCGGCCC  
CACTTCCTCACCCAGCTCCACTCCACCTTCCAGACCCCGGACCGCCTG  
TATTTCGTGATGGAGTACGTACCGGGGAGACTTGATGTACCACATT  
CAACAGCTGGGCAAGTTTAAGGAGCCCCATGCAGCGTTCTACGCGGCA  
GAAATCGCTATCGGCCTCTTCTTCCTTCACAATCAGGGCATCATCTAC  
AGGGACCTGAAGCTGGACAATGTGATGCTGGATGCTGAGGGACACATC  
AAGATCACTGACTTTGGCATGTGTAAGGAGAACGTCTTCCCCGGGACG  
ACAACCCGCACCTTCTGCGGGACCCCGGACTACATAGCCCCGGAGATC  
ATTGCCTACCAGCCCTATGGGAAGTCTGTGCGATTGGTGGTCCTTTGGA  
GTTCTGCTGTATGAGATGTTGGCAGGACAGCCTCCCTTCGATGGGGAG  
GACGAGGAGGAGCTGTTTCAGGCCATCATGGAACAACTGTCACCTAC  
CCCAAGTCGCTTTCCCGGGAAGCCGTGGCCATCTGCAAGGGGTTCTTG  
ACCAAGCACCCAGGGAAGCGCCTGGGCTCAGGGCCTGATGGGGAACCT  
ACCATCCGTGCACATGGCTTTTTTCCGCTGGATTGACTGGGAGCGGCTG  
GAACGATTGGAGATCCCGCCTCCTTTTCAGACCCCGCCGCTGTGGCCGC  
AGCGGCGAGAACTTTGACAAGTTCTTCACGCGGGCGGCGCCAGCGCTG  
ACCCCTCCAGACCGCCTAGTCCTGGCCAGCATCGACCAGGCCGATTTTC  
CAGGGCTTCACCTACGTGAACCCCGACTTCGTGCACCCGGATGCCCCG  
AGCCCCACCAGCCCAGTGCCTGTGCCCCGTATGtaagcgccgc