

# *MRC PPU REAGENTS*

## Standard Operating Procedure

### Preparation of active Protein Kinase C beta isoform 2 [1 – 673]

<b><u>Enzyme description:-</u></b>	PRKCB [1 - 673] isoform 2
<b><u>Clone number:-</u></b>	DU 33630
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6)
<b><u>Purification method:-</u></b>	Cobalt agarose

**Calculated molecular mass:-**

Monoisotopic      80, 331.58 daltons  
Average Mass      80, 383.74 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      6.45

**Purity:-**                                      85 %

**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, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-**                      -70 °C

**Assay buffer:-**

50 mM Tris-HCl pH 7.5, 0.1 mM CaCl<sub>2</sub>, 0.1 mg/ml PtdSerine, 10 uM DAG, 10 mM DTT,  
10 mM Magnesium Acetate

**Substrate:-**

ERM<sup>R</sup>PRKRQGSVRRV                      Final concentration: 300 uM

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## Clone Data Sheet

### PKC beta [1 - 673] isoform 2

**Protein** PRKCB [1 - 673] isoform 2

**Clone number** DU 33630

**Species** Human

**Accession number** NM\_002738.7

**Tags** N-terminal His(6)

**Baculovirus  
expressed protein**

MSYYHHHHHDYDIPTTENLYFQ**GAMGSMADPAAGPPPSEGEESTVRFA**  
**RKGALRQKNVHEVKNHKFTARFFKQPTFC**SHCTDFIWFGKQGFQ**QCQVC**  
**CFVVKRCHEFVTF**SCPGADKGPASDDPRSKHKFKIHTYSSPT**CDHCG**  
**SLLYGLIHQGMKCDT**CMNVHKRCVMNVPSLC**GTDHTERRGRIYIQAH**  
**DRDVLIVLVRDAK**NLVPMDPNGLSDPYV**KLKLI**PDPK**SESKQKTKTIK**  
**SLNPEWNETFRFQ**LKESDKDRRLSVEIWD**DLTSR**NDFMGSL**SFGISEL**  
**QKASVDGWFKLLSQ**EEGEYFNVPVP**PEGSEANEELRQK**FERAKIS**QGTK**  
**VPEEKTNTVSKF**DNNGNRDRM**KLTD**FNFLMV**L**GKGSFGK**VMLSERKGT**  
**DELYAVKILK**KDVVIQDDDV**ECTMVEKRVLAL**PGKPP**FLTQLHSCFQTM**  
**DRLYFVMEYV**NGDLMYHIQ**QVGRFKEPHAVFYAAE**IAIGL**FFLQSKGI**  
**IYRDLKLDN**VMLDSEGH**IKIAD**FGMCKENIWDG**VTTKTF**CGTPDY**IAPE**  
**I IAYQPYGKS**VDNWAFGVLLY**EMLAGQAP**FEGEDE**DEL**FQSIMEH**NVAY**  
**PKSMSKEAVA**ICKGLMT**KHPGKRLGCG**PEGERDIKEHA**FFRYID**WEKLE  
**RKEIQPPYKPKAC**GRNAEN**DRFFTRHPPVLT**PPDQEVIR**NIDQSEFEG**  
**FSFVNSEFLKPEVKS**

**Native sequence** Amino acids M1 – S673 (end) of human PRKCB isoform 2.  
Residue M29 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.

**Protease cleavage** rTEV (ENLYFQ) residues 18 - 23

**Cloning sites** *Bam*H1 and *Sal*I sites of pFastBac.Htb

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### Nucleotide Sequence Of Insert

ggatccATGGCTGACCCGGCTGCGGGGCCGCCGCCGAGCGAGGGCGAGGAGAGCACCGTGCGCTTC  
GCCCCGAAAGGCGCCCTCCGGCAGAAGAACGTGCATGAGGTCAAGAACCACAAATTCACCGCCCGC  
TTCTTCAAGCAGCCCACCTTCTGCAGCCACTGCACCGACTTCATCTGGGGCTTCGGGAAGCAGGGA  
TTCCAGTGCCAAGTTTGCTGCTTTGTGGTGCACAAGCGGTGCCATGAATTTGTACATTCTCCTGC  
CCTGGCGCTGACAAGGGTCCAGCCTCCGATGACCCCCGAGCAAACACAAGTTTAAGATCCACACG  
TACTCCAGCCCCACGTTTTGTGACCCTGTGGGTCACTGCTGTATGGACTCATCCACCAGGGGATG  
AAATGTGACACCTGCATGATGAATGTGCACAAGCGCTGCGTGATGAATGTTCCAGCCTGTGTGGC  
ACGGACCACACGGAGCGCCGCGGCCGCATCTACATCCAGGCCACATCGACAGGGACGTCCCTCATT  
GTCCTCGTAAGAGATGCTAAAAACCTTGTACCTATGGACCCCAATGGCCTGTCAGATCCCTACGTA  
AAACTGAAACTGATTCCCGATCCCAAAAGTGAGAGCAAACAGAAGACCACAAACCATCAAATGCTCC  
CTCAACCCTGAGTGGAATGAGACATTTAGATTTTCAGCTGAAAGAATCGGACAAAGACAGAAGACTG  
TCAGTAGAGATTTGGGATTTGGGATTTGACCAGCAGGAATGACTTCATGGGATCTTTGTCTTTGGG  
ATTTCTGAACCTCAGAAAGCCAGTGTGATGGCTGGTTTTAAGTTACTGAGCCAGGAGGAAGGCGAG  
TACTTCAATGTGCCTGTGCCACCAGAAGGAAGTGAGGCCAATGAAGAAGTGCGGCAGAAATTTGAG  
AGGGCCAAGATCAGTCAGGGAACCAAGTCCCAGGAAAGAACGACCAACACTGTCTCCAAATTT  
GACAACAATGGCAACAGAGACCAGGATGAAACTGACCGATTTTAACTTCCTAATGGTGCTGGGGAAA  
GGCAGCTTTGGCAAGGTCATGCTTTTCAGAACGAAAAGGCACAGATGAGCTCTATGCTGTGAAGATC  
CTGAAGAAGGACGTTGTGATCCAAGATGATGACGTGGAGTGCACATGTTGGGAGGAGCGGGTGTG  
GCCCTGCCTGGGAAGCCGCCCTTCCTGACCCAGCTCCACTCCTGCTTCCAGACCATGGACCGCCTG  
TACTTTGTGATGGAGTACGTGAATGGGGGCGACCTCATGTATCACATCCAGCAAGTCGGCCGGTTC  
AAGGAGCCCCATGCTGTATTTTACGCTGCAGAAATTGCCATCGGTCTGTTCTTCTTACAGAGTAAG  
GGCATCATTTACCGTGACCTAAAACCTTGACAACGTGATGCTCGATTCTGAGGGACACATCAAGATT  
GCCGATTTTGGCATGTGTAAGGAAAACATCTGGGATGGGGTGACAACCAAGACATTCGTGTGGCACT  
CCAGACTACATCGCCCCGAGATAATTGCTTATCAGCCCTATGGGAAGTCCGTGGATTTGGTGGGCA  
TTTGGAGTCCTGCTGTATGAAATGTTGGCTGGGCAGGCACCCTTTGAAGGGGAGGATGAAGATGAA  
CTCTTCCAATCCATCATGGAACACAACGTAGCCTATCCAAGTCTATGTCCAAGGAAGCTGTGGCC  
ATCTGCAAAGGGCTGATGACCAAACACCCAGGCAAACGTCTGGGTTGTGGACCTGAAGGCGAACGT  
GATATCAAAGAGCATGCATTTTCCGGTATATGATTGGGAGAACTTGAACGCAAAGAGATCCAG  
CCCCCTTATAAGCCAAAAGCTTGTGGGCGAAAATGCTGAAAACCTTCGACCGATTTTTACCCGCCAT  
CCACCAGTCCTAACACCTCCCGACCAGGAAGTCATCAGGAATATTGACCAATCAGAATTCGAAGGA  
TTTTCTTTGTTAACTCTGAATTTTTTAAAACCCGAAGTCAAGAGCtaagtcgac