

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of active Protein Kinase C epsilon [1 – 737]

<u>Enzyme description:-</u>	PRKCE [1 - 737]
<u>Clone number:-</u>	DU 33642
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Cobalt agarose

Calculated molecular mass:-

Monoisotopic 86, 989.48 daltons
Average Mass 87, 045.42 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.54

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 CaCl₂, 0.1 mg/ml PtdSerine, 10 uM DAG, 10 mM DTT,
10 mM Magnesium Acetate

Substrate:-

ERM^RPRKRQGSVRRV Final concentration: 300 uM

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Clone Data Sheet

PKC epsilon [1 - 737]

Protein PRKCE [1 - 737]

Clone number DU 33642

Species Human

Accession number NM_005400.3

Tags N-terminal His(6)

**Baculovirus
expressed protein**

MSYYHHHHHDYDIPTTENLYFQAGAMGSMVVFNGLLKIKICEAVSLKPT
AWSLRHAVGPRPQTFLLDPYIALNVDDSRIGQTATKQKTNSPAWHDEFV
TDVCNGRKIELAVFHDAPIGYDDFVANCTIQFEELLONGSRHFEDWIDL
EPEGRVYVIIDLSGSSGEAPKDNEERVFRERMRPRKRQGAVRRRVHQVN
GHKFMATYLRQPTYCSHCRDFIWGVI GKQGYQCQVCTCVVHKRCHELII
TKCAGLKKQETPDQVGSQRF SVNMPHKFGIHNKVPPTFCDHCGSLLWGL
LRQGLQCKVCKMNVHRR CETNVAPNCGVDARGIAKVLADLGVTPDKITN
SGQRRKKLIAGAESPQPASGSSPSEEDRSKSAPTSPCDQEI KELENNIR
KALSFDNRGEEHRAASSPDGQLMSPGENGEVVRQQAQRLGLDEFNFIKV
LGKGSFGKVMLAELK GKDEVYAVKVLKDVILQDDVDCTMTEKRILAL
ARKHPYLTQLYCCFQTKDRLFFVMEYVNGGDLMFQIQRSRKFDEPRSRF
YAAEVTSALMFLHQHGV IYRDLKLDNILLDAEGHCKLADFGMCKEGILN
GVTTTTFCGTPDYIAPEILQELEYGPSVDWWALGVL MYEMMAGQPPFEA
DNEDDLFESILHDDVLYPVWLSKEAVSILKAFMTKNPHKRLGCVASQNG
EDA IKQHPFFKEIDWV LLEQKKIKPPFKPRIKTRKRDVNNFDQDF TREEP
VLT LVDEAIVKQINQEEFKGFSYFGEDLMP

Native sequence Amino acids M1 – P737 (end) of human PRKCE.
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 *EcoR1* and *Not1* sites of pFastBac.Hta

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Nucleotide Sequence Of Insert

ggatccATGGTAGTGTTC AATGGCCTTCTTAAGATCAAAATCTGCGAGGCCGTGAGCTTGAAGCCC
ACAGCCTGGTCGCTGCGCCATGCGGTGGGACCCCGGCCGACACTTTCCTTCTCGACCCCTACATT
GCCCTCAATGTGGACGACTCGCGCATCGGCCAAACGGCCACCAAGCAGAAGACCAACAGCCCGGCC
TGGCACGACGAGTTCGTCACCGATGTGTGCAACGGACGCAAGATCGAGCTGGCTGTCTTTCACGAT
GCCCCATAGGCTACGACGACTTCGTGGCCAACTGCACCATCCAGTTTGAGGAGCTGCTGCAGAAC
GGGAGCCGCCACTTCGAGGACTGGATTGATCTGGAGCCAGAAGGAAGAGTGTATGTGATCATCGAT
CTCTCAGGGTCGTCGGGTGAAGCCCCTAAAGACAATGAAGAGCGTGTGTTTCAGGGAACGCATGCGG
CCGAGGAAGCGGCAGGGGGCCGTCAGGCGCAGGGTCCATCAGGTCAACGGCCACAAGTTCATGGCC
ACCTATCTTCGGCAGCCACCTACTGCTCCCATTTGCAGAGACTTCATCTGGGGTGTGCATAGGAAAG
CAGGGATACCAGTGTCAAGTCTGCACCTGCGTGGTCCACAAGCGGTGCCACGAGCTCATAATCACA
AAGTGTGCTGGGTAAAGAAGCAGGAGACCCCGACCAGGTGGGCTCCAGCGGTTTCAGCGTCAAC
ATGCCCCACAAGTTCGGTATCCACAAC TACAAGTCCCTACCTTCTGCGATCACTGTGGGTCCCTG
CTCTGGGGACTCTTGC GGCAGGGTTTTGCAGTGTAAGTCTGCAAATGAATGTTACCGTCGATGT
GAGACCAACGTGGCTCCCAACTGTGGAGTGGATGCCAGAGGAATCGCCAAAGTACTGGCCGACCTG
GGCGTTACCCAGACAAAATCACC AACAGCGGCCAGAGAAGGAAAAAGCTCATTGCTGGTGCCGAG
TCCCCGCAGCCTGCTTCTGGAAGCTCACCATCTGAGGAAGATCGATCCAAGTCAGCACCCACCTCC
CCTTGTGACCAGGAAATAAAAGAACTTGAGAACAACATTCGAAAGCCTTGTCAATTTGACAACCGA
GGAGAGGAGCACCGGGCAGCATCGTCTCCTGATGGCCAGCTGATGAGCCCCGGTGAGAATGGCGAA
GTCCGGCAAGGCCAGGCCAAGCGCCTGGGCTGGATGAGTTCAACTTCATCAAGGTGTTGGGCAA
GGCAGCTTTGGCAAGGTCATGTTGGCAGA ACTCAAGGGCAAAGATGAAGTATATGCTGTGAAGGTC
TTAAAGAAGGACGTCATCCTTCAGGATGATGACGTGGACTGCACAATGACAGAGAAGAGGATTTTG
GCTCTGGCACGGAAACACCCGTACCTTACCCA ACTCTACTGCTGCTTCCAGACCAAGGACCGCCTC
TTTTTCGTCATGGAATATGTAAATGGTGGAGACCTCATGTTTCAGATTCAGCGCTCCCGAAAATTC
GACGAGCCTCGTTCACGGTTCATGCTGCAGAGGTCACATCGGCCCTCATGTTCTCCTCACCAGCAT
GGAGTCATCTACAGGGATTTGAAACTGGACAACATCCTTCTGGATGCAGAAGGTC ACTGCAAGCTG
GCTGACTTCGGGATGTGCAAGGAAGGGATTCTGAATGGTGTGACGACCACCACGTTCTGTGGGACT
CCTGACTACATAGCTCCTGAGATCCTGCAGGAGTTGGAGTATGGCCCCTCCGTGGACTGGTGGGCC
CTGGGGGTGCTGATGTACGAGATGATGGCTGGACAGCCTCCCTTTGAGGCCGACAATGAGGACGAC
CTATTTGAGTCCATCCTCCATGACGACGTGCTGTACCCAGTCTGGCTCAGCAAGGAGGCTGT CAGC
ATCTTGAAAGCTTTCATGACGAAGAATCCCCACAAGCGCCTGGGCTGTGTGGCATCGCAGAATGGC
GAGGACGCCATCAAGCAGCACCCATTCTTCAAAGAGATTGACTGGGTGCTCCTGGAGCAGAAGAAG
ATCAAGCCACCCTTCAAACCACGCATTA AAAACCAAAGAGACGTCAATAATTTTGACCAAGACTTT
ACCCGGGAAGAGCCGGTACTCACCTTGTGGACGAAGCAATTGTAAAGCAGATCAACCAGGAGGAA
TTCAAAGGTTTTCTCCTACTTTGGTGAAGACCTGATGCCCTgagcggccgc