

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of active Protein Kinase C eta [1 – 683]

<u>Enzyme description:-</u>	PRKCH [1 - 683]
<u>Clone number:-</u>	DU 29921
<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 81, 491.54 daltons
Average Mass 81, 544.45 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.03

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 eta [1 - 683]

Protein PRKCH [1 - 683]

Clone number DU 29921

Species Human

Accession number NM_006255.4

Tags N-terminal His(6)

**Bacterially
expressed protein**

MSYYHHHHHDYDIPTTENLYFQGAMDPEFMSSGTMKFNGLRVRI GEA
VGLQPTRWSLRHSLFKKGHQLLDPYLTVSVDQVRVGQTSTKQKTNKPTY
NEEFCANVTDGGHLELAVFHETPLGYDHFVANCTLOFQELLRTTGASDT
FEGWVDLEPEGKVFVVIITLGSFTEATLQRDRIFKHFTKRQRAMRRRV
HQINGHKFMATYLRQPTYCSHCREFIWGVFGKQGYQCQVCTCVVHKRCH
HLIVTACTCQNNINKVDSKIAEQRFGINIPHKFSIHNYKVPTFCDHCGS
LLWIMRQGLQCKICKMNVHIRCQANVAPNCGVNAVELAKTLAGMGLQP
GNISPTSKLVSRSSTLRRQKESSEKENGIGVNSSNRLGIDNFEFIRVLG
KGSFGKVMLARVKETGDLAVKVLKGDVILQDDVECTMTEKRILSLAR
NHPFLTQLFCCFQTPDRLFFVMEFVNGGDLMFHIQKSRRFDEARARFYA
AEIISALMFLHDKGIYRDLKLDNVLLDHEGHCKLADFGMCKEGICNGV
TTATFCGTPDYIAPEILQEMLYGPAVDWAMGVLLYEMLCGHAPFEAEN
EDDLFEAILNDEVVYPTWLHEDATGILKSFMTKNPTMRLGSLTQGEHA
ILRHPPFFKEIDWAQLNHRQIEPPFRPRIKSREDVSNFDPDFIKEEPVLT
PIDEGHLPMINQDEFNRNFSYVSPELOP

Native sequence Amino acids M1 – P683 (end) of human PRKCH.
Residue M31 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

gaattcATGTCGTCTGGCACCATGAAGTTCAATGGCTATTTGAGGGTCCG
CATCGGTGAGGCAGTGGGGCTGCAGCCACCCGCTGGTCCCTGCGCCACT
CGCTCTTCAAGAAGGGCCACCAGCTGCTGGACCCCTATCTGACGGTGAGC
GTGGACCAGGTGCGCGTGGGCCAGACCAGCACCAAGCAGAAGACCAACAA
ACCCACGTACAACGAGGAGTTTTGCGCTAACGTCACCGACGGCGGCCACC
TCGAGTTGGCCGTCTTCCACGAGACGCCCTGGGCTACGACCACCTTCGTG
GCCAACTGCACCCTGCAGTTCAGGAGCTGCTGCGCACGACCGGCGCCTC
GGACACCTTCGAGGGTTGGGTGGATCTCGAGCCAGAGGGGAAAGTATTTG
TGTAATAACCCTTACCGGGAGTTTCACTGAAGCTACTCTCCAGAGAGAC
CGGATCTTCAAACATTTTACCAGGAAGCGCCAAAGGGCTATGCGAAGGCG
AGTCCACCAGATCAATGGACACAAGTTCATGGCCACGTATCTGAGGCAGC
CCACCTACTGCTCTCACTGCAGGGAGTTTATCTGGGGAGTGTTTGGGAAA
CAGGGTTATCAGTGCCAAGTGTGCACCTGTGTCGTCCATAAACGCTGCCA
TCATCTAATTGTTACAGCCTGTACTTGCCAAAACAATATTAACAAAGTGG
ATTCAAAGATTGCAGAACAGAGGTTTCGGGATCAACATCCCACACAAGTTC
AGCATCCACAAC TACAAAGTGCCAACATTTCTGCGATCACTGTGGCTCACT
GCTCTGGGGAATAATGCGACAAGGACTTCAGTGTA AAAATATGTAAAATGA
ATGTGCATATTCGATGTCAAGCGAACGTGGCCCCTAACTGTGGGGTAAAT
GCGGTGGAAC TTGCCAAGACCC TGGCAGGGATGGGTCTCCAACCCGAAA
TATTTCTCCAACCTCGAAACTCGTTTCCAGATCGACCCTAAGACGACAGG
GAAAGGAGAGCAGCAAAGAAGGAAATGGGATTGGGGTTAATTCTTCCAAC
CGACTTGGTATCGACAAC TTTGAGTTCATCCGAGTGTGGGGAAGGGGAG
TTTTGGGAAGGTGATGCTTGCAAGAGTAAAAGAAACAGGAGACCTCTATG
CTGTGAAGGTGCTGAAGAAGGACGTGATTCTGCAGGATGATGATGTGGAA
TGCACCATGACCGAGAAAAGGATCCTGTCTCTGGCCC GCAATCACCCCTT
CCTCACTCAGTTGTTCTGCTGCTTTTCAGACCCCGATCGTCTGTTTTTTG
TGATGGAGTTTGTGAATGGGGGTGACTTGATGTTCCACATTCAGAAGTCT
CGTCGTTTTGATGAAGCACGAGCTCGCTTCTATGCTGCAGAAATCATTTT
GGCTCTCATGTTCTCCATGATAAAGGAATCATCTATAGAGATCTGAAAC
TGGACAATGTCTGTTGGACCACGAGGGTCACTGTAAACTGGCAGACTTC
GGAATGTGCAAGGAGGGGATTTGCAATGGTGTCAACACGGCCACATTCTG
TGGCACGCCAGACTATATCGCTCCAGAGATCCTCCAGGAAATGCTGTACG
GGCCTGCAGTAGACTGGTGGGCAATGGGCGTGTGCTCTATGAGATGCTC
TGTGGTCACGCGCCTTTTGAGGCAGAGAATGAAGATGACCTCTTTGAGGC
CATACTGAATGATGAGGTGGTCTACCC TACCTGGCTCCATGAAGATGCCA
CAGGGATCCTAAAATCTTTCATGACCAAGAACCCACCATGCGCTTGGGC
AGCCTGACTCAGGGAGGCGAGCACGCCATCTTGAGACATCCTTTTTTTTAA
GGAAATCGACTGGGCCAGCTGAACCATCGCCAAATAGAACC GCCTTTCA
GACCCAGAATCAAATCCCGAGAAGATGTCAGTAATTTTGACCCTGACTTC
ATAAAGGAAGAGCCAGTTTTAACTCCAATTGATGAGGGACATCTTCCAAT
GATTAACCAGGATGAGTTTTAGAACTTTTCTATGTGTCTCCAGAATTGC
AACCAtaggcggccgc