

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PKC alpha [1 - 672]

<u>Enzyme description:-</u>	PKC alpha [1 - 672]
<u>Clone number:-</u>	DU 5084
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	8 mg/L

Calculated molecular mass:-

Monoisotopic	80, 427.69 daltons
Average Mass	80, 479.95 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.33

Purity:- > 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

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

Storage temperature:- -70 °C [Long term stability to be determined]

Assay:- Standard filter binding assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc, 0.1 mM CaCl₂ 0.1 mg/ml PtdSerine, 10 µg/m DAG

Substrate:-

Histone H1 Final concentration: 0.1 mg/ml

Specific activity range:- To be determined

Division of Signal Transduction Therapy

Clone Data Sheet

PKC alpha [1 - 672]

<u>Protein</u>	PKC alpha [1 - 672]
<u>Clone number</u>	DU 5084
<u>Species</u>	Human
<u>Accession number</u>	NM_002737
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQGAMDPEFMADVFPGNDSTASQDVANR FARKGALRQKNVHEVKDHKFIARFFKQPTFC SHCTDFIWGFGKQGFQCQ VCCFVVHKRCHEFVTFSCPGADKGPDTDDPRSKHKFKIHTYGSPTFCDH CGSLLYGLIHQGMKCDTCDMNVHKQCVINVPSLCGMDHTEKRGRIYLKA EVADEKLHVTVRDAKNLIPMDPNGLSDPYVKLKLIPDPKNESKQKTKTI RSTLNPQWNESFTFKLKPSDKDRRLSVEIWDWDRTRNDFMGSLSFVGS ELMKMPASGWYKLLNQEEGEYYNVPIPEGDEEGNMELRQKF EKAKLGPA GNKVISPSEDRKQPSNNLDRVKLTDNFNLMVLGKGSFGKVMLADRKGTE ELYAIKILKKDVVIQDDDV ECTMVEKRVLALLDKPPFLTQLHSCFQTVD RLYFVMEYVNGGDLMYHIQQVGKFKEPQAVFYAAEISIGLFFLHKRGII YRDLKLDNVMLDSEGHIKIADFGMCKEHMMDGVTTRTFCGTPDYIAPEI IAYQPYGKSVDWWAYGVLLYEMLAGQPPFDGEDEDEL FQSIMEHNVSYP KSLSKEAVSICKGLMTKHPAKRLGCGPEGERDVREHAFFRRIDWEKLEN REIQPPFKPKVCGGAENFDKFFTRGQPVLTPPDQLVIANIDQSDFE GF SYVNPQFVHPILQSAV
<u>Native sequence</u>	Amino acids M1 – V672 (end) of human PKC alpha. Residue M31 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 - 24
<u>Cloning sites</u>	<i>Eco</i> R1 and <i>Not</i> I site in pFastBAC HTa

Division of Signal Transduction Therapy

Nucleotide
sequence of insert

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGATCCGGAATTCATGGCTGA
CGTTTTCCCGGGCAACGACTCCACGGCGTCTCAGGACGTGGCCAACCGC
TTCGCCCCGAAAGGGGCGCTGAGGCAGAAGAACGTGCACGAGGTGAAGG
ACCACAAATTCATCGCGCGCTTCTTCAAGCAGCCACCTTCTGCAGCCA
CTGCACCGACTTCATCTGGGGGTTTGGGAAACAAGGCTTCCAGTGCCAA
GTTTGCTGTTTTGTGGTCCACAAGAGGTGCCATGAATTTGTTACTTTTT
CTTGTCCGGGTGCGGATAAGGGACCCGACACTGATGACCCAGGAGCAA
GCACAAGTTCAAAATCCACACTTACGGAAGCCCCACCTTCTGCGATCAC
TGTGGGTCACTGCTCTATGGACTTATCCATCAAGGGATGAAATGTGACA
CCTGCGATATGAACGTTCAACAAGCAATGCGTCATCAATGTCCCCAGCCT
CTGCGGAATGGATCACACTGAGAAGAGGGGGCGGATTTACCTAAAGGCT
GAGTTTGCTGATGAAAAGCTCCATGTACAGTACGAGATGCAAAAAATC
TAATCCCTATGGATCCAAACGGGCTTTCAGATCCTTATGTGAAGCTGAA
ACTTATTCCTGATCCCAAGAATGAAAGCAAGCAAAAAACCAAAACCATC
CGCTCCACACTAAATCCGCAGTGGAAATGAGTCCTTTACATTCAAATTGA
AACCTTCAGACAAAGACCGACGACTGTCTGTAGAAATCTGGGACTGGGA
TCGAACAACAAGGAATGACTTCATGGGATCCCTTTCTTTGGAGTTTCG
GAGCTGATGAAGATGCCGGCCAGTGGATGGTACAAGTTGCTTAACCAAG
AAGAAGGTGAGTACTACAACGTACCCATTCCGGAAGGGGACGAGGAAGG
AAACATGGAACCTCAGGCAGAAATTCGAGAAAGCCAAACTTGCCCTGCT
GGCAACAAGTCATCAGTCCCTCTGAAGACAGGAAACAACCTTCCAACA
ACCTTGACCGAGTGAACTCACGGACTTCAATTTCTCATGGTGTGGG
AAAGGGGAGTTTTGGAAAGGTGATGCTTGCCGACAGGAAGGGCACAGAA
GAACTGTATGCAATCAAAATCCTGAAGAAGGATGTGGTGATTCAGGATG
ATGACGTGGAGTGCACCATGGTAGAAAAGCGAGTCTTGCCCTGCTTGA
CAAACCCCGTTCTTGACGCAGCTGCACTCCTGCTTCCAGACAGTGGAT
CGGCTGTACTTCGTATGGAATATGTCAACGGTGGGGACCTCATGTACC
ACATTCAAGTAGGAAAATTTAAGGAACCACAAGCAGTATTCTATGC
GGCAGAGATTTCCATCGGATTGTTCTTTCTTCATAAAAGAGGAATCATT
TATAGGGATCTGAAGTTAGATAACGTCATGTTGGATTCAGAAGGACATA
TCAAAATTGCTGACTTTGGGATGTGCAAGGAACACATGATGGATGGAGT
CACGACCAGGACCTTCTGTGGGACTCCAGATTATATCGCCCCAGAGATA
ATCGCTTATCAGCCGTATGGAAAATCTGTGGACTGGTGGGCCTACGGCG
TCCTGTTGTATGAAATGCTTGCCGGGCAGCCTCCATTTGATGGTGAAGA
TGAAGACGAGCTATTTCACTATCATGGAGCACAAACGTTTCCTATCCA
AAATCCTTGTCCAAGGAGGCTGTTTCTATCTGCAAAGGACTGATGACCA
AACACCCAGCCAAGCGGCTGGGCTGTGGGCCTGAGGGGGAGAGGGACGT
GAGAGAGCATGCCTTCTTCCGGAGGATCGACTGGGAAAACTGGAGAAC
AGGGAGATCCAGCCACCATTCAAGCCCAAAGTGTGTGGCAAAGGAGCAG
AGAAGTTTGACAAGTTCTTACACGAGGACAGCCCGTCTTAACACCACC
TGATCAGCTGGTTATTGCTAACATAGACCAGTCTGATTTTGAAGGGTTC
TCGTATGTCAACCCCAAGTTTGTGCACCCCAT
CTTACAGAGTGCAAGTga