Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active PDPC2 [2 - 529] (Pyruvate Dehydrogenase Phosphatase Isoenzyme 2)

Enzyme description:- PDPC2 [2 - 529]

Clone number:- DU 1363

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:- 86, 615 daltons

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 2 mM $MnCl_2$, 0.03 % Brij 35, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 50 % glycerol, 1 mM benzamidine and 0.1 mM PMSF

Storage temperature:- -20 °C

Assay:- Standard phosphatase assay

Assay buffer:-

50 mM Tris-HCl pH 7.5, 2 mM $MnCl_2$, 0.03 % Brij 35, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol

Substrate:-

6 µM ^{32}P labelled casein (phosphorylated by PKA)

Specific activity range:- To be determined

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Clone Data Sheet

PDPC2 [2 – 529]

Protein	PDPC2 [2 – 529]
Clone number	DU 1363
Species	Human
Accession number	BC028030
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**SSTVSYWILNSTRNSIATL QGGRRLYSRYVSNRNKLKWRLFSRVPPTLNSSPCGGFTLCKAYRHTSTEE DDFHLQLSPEQINEVLRAGETTHKILDLESRVPNSVLRFESNQLAANSPV EDRRGVASCLQTNGLMFGIFDGHGGHACAQAVSERLFYYVAVSLMSHQTL EHMEGAMESMKPLLPILHWLKHPGDSIYKDVTSVHLDHLRVYWQELLDLH MEMGLSIEEALMYSFQRLDSDISLEIQAPLEDEVTRNLSLQVAFSGATAC MAHVDGIHLHVANAGDCRAILGVQEDNGMWSCLPLTRDHNAWNQAELSRL KREHPESEDRTIIMEDRLLGVLIPCRAFGDVQLKWSKELQRSILERGFNT EALNIYQFTPPHYYTPPYLTAEPEVTYHRLRPQDKFLVLASDGLWDMLSN EDVVRLVVGHLAEADWHKTDLAQRPANLGLMQSLLLQRKASGLHEADQNA ATRLIRHAIGNNEYGEMEAERLAAMLTLPEDLARMYRDDITVTVVYFNSE SIGAYYKGG**
Native sequence	Amino acids S2 – G529 (end) of human PDPC2. Residue S232 of the fusion protein is equivalent to G2 of the native enzyme The GST tag is located at residues 1 - 220.
Protease cleavage	PreScission (LEVLFQGPL) at residues 221 - 229
Cloning sites	*Bam*H1 and *Not*I sites of pGEX-6P-1

Nucleotide sequence of insert

ggatccTCAAGTACTGTGTCCTACTGGATCTTAAATTCTACAA
GGAACAGCATTGCCACATTGCAAGGGGGTAGACGCTTATACTC
CAGGTATGTCTCAAATAGGAATAAATTAAAATGGAGGCTCTTT
TCCCGGGTGCCACCCACCCTAAACAGTTCCCCATGTGGTGGCT
TTACTCTGTGCAAAGCCTACAGACACACATCAACAGAGGAAGA
TGATTTTCACTTGCAACTCAGCCCTGAGCAGATAAATGAAGTG
CTTCGAGCTGGCGAGACAACCCACAAGATTCTTGACCTTGAAA
GCAGAGTCCCAAATTCAGTGTTGCGGTTTGAGAGCAACCAGCT
GGCTGCCAATTCCCCAGTGGAGGACCGGCGAGGTGTAGCCTCC
TGCCTGCAAACCAATGGACTGATGTTTGGCATCTTCGATGGAC
ATGGTGGTCATGCATGTGCCCAAGCAGTGAGCGAGAGGCTCTT
CTACTATGTGGCAGTGTCCCTGATGTCCCACCAGACCCTGGAG
CACATGGAGGGAGCTATGGAAAGCATGAAACCCTTGCTGCCCA
TCCTGCATTGGCTCAAGCACCCAGGGGACAGTATCTACAAGGA
TGTCACATCTGTGCATCTTGACCACCTCCGTGTCTATTGGCAG
GAACTGCTTGATTTGCACATGGAAATGGGACTAAGCATTGAAG
AAGCATTAATGTACTCCTTCCAGAGACTGGATTCTGACATCTC
GCTGGAAATCCAGGCCCCCCTGGAAGATGAGGTGACAAGGAAC
CTGTCACTCCAGGTTGCTTTCTCTGGGGCAACAGCTTGCATGG
CCCATGTTGATGGAATTCACTTGCACGTGGCAAATGCTGGCGA
CTGCCGAGCCATCCTTGGTGTCCAAGAGGACAATGGCATGTGG
TCTTGTCTGCCCCTTACACGTGACCACAATGCCTGGAACCAGG
CCGAGCTGTCCCGGCTAAAGAGGGAGCACCCTGAGTCAGAGGA
CAGGACGATCATCATGGAGGACAGGCTACTGGGCGTCCTCATC
CCCTGCAGGGCCTTTGGGGATGTTCAGCTGAAGTGGAGTAAAG
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CCTCAACATTTACCAGTTCACACCCCCACACTACTACACTCCA
CCCTACCTGACTGCTGAGCCTGAGGTCACATACCACAGGCTGA
GGCCCCAGGATAAGTTCCTTGTGCTGGCCTCAGATGGCCTGTG
GGACATGCTGAGCAATGAGGACGTGGTAAGGCTGGTGGTGGGG
CACCTGGCTGAGGCAGATTGGCACAAGACAGACCTGGCCCAGA
GACCCGCCAACTTGGGGCTCATGCAGAGCCTGCTGCTGCAGAG
GAAAGCCAGCGGGCTCCACGAGGCTGACCAAAATGCAGCCACG
CGGCTGATCAGACATGCCATCGGGAACAATGAGTATGGGGAGA
TGGAGGCAGAGCGGCTGGCGGCGATGCTGACATTGCCAGAGGA
CTTGGCGAGGATGTACAGGGATGATATCACTGTCACTGTGGTG
TATTTTAACTCAGAATCAATCGGTGCATATTACAAGGGGGGTt
aagcggccgc