

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PP2C alpha [1 – 382]

Enzyme description:- PP2C alpha [1 - 382]

Clone number:- DU 1852

Source:- Recombinant

Expression system:- *E.coli*

Tag:- None

Purification method:-

30 – 55 % ammonium sulphate precipitation, Q-Sepharose and Mono-Q

Method based on:

Davis, SP. Helps, NR. Cohen, PTW. and Hardie, DG. (1995).

“5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase 2C alpha and native bovine protein phosphatase 2A_c”

FEBS Letters. 377. 421 - 425.

Expression level:- 3 mg/L

Calculated molecular mass:- 42, 420 daltons

Purity:- >90 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 2 mM MnCl₂, 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 MnCl₂, 0.03 % Brij 35, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol

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Substrate:-

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

Specific activity range:-

80 – 160 mU/mg

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Clone Data Sheet

PP2C alpha [1 - 382]

<u>Protein</u>	PP2C alpha [1 - 382]
<u>Clone number</u>	DU 1852
<u>Species</u>	Human
<u>Accession number</u>	S87759
<u>Tags</u>	None
<u>Bacterially expressed protein</u>	<p>MGAFLDKPKMEKHNAQGQGNGLRYGLSSMQGWRVEMEDAHTAVI GLPSGLESWSFFAVYDGHAGSQVAKYCCEHLLDHIITNNQDFKGS AGAPSVENVKNGIRTGFLEIDEHMRVMSEKKHGADRSGSTAVGV LISPQHTYFINCGDSRGLLCRNRKVHFFTQDHKPSNPLEKERIQ NAGGSVMIQRVNGSLAVSRALGDFDYKCVHGKGPTEQLVSPEPE VHDIERSEEDDQFIILACDGIWDVMGNEELCDFVRSRLEVTTDDL EKVCNEVVDTCLYKGSRDNMSVILICFPNAPKVSPEAVKKEAEL DKYLECRVEEIIKKQGEVVDLVHVMRTLASENIPSLPPGGELA SKRNVIEAVYNRLNPYKNDDTDSTSTDDMW</p>
<u>Native sequence</u>	Amino acids M1 – W382 (end) of human PP2C alpha. M1 is the initiating methionine of PP2C alpha
<u>Protease cleavage</u>	None
<u>Cloning sites</u>	<i>Nde</i> I and <i>Hind</i> III sites of pCW

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**Nucleotide
sequence of insert**

ATGGGAGCATTTTTGTAGACAAGCCAAAGATGGAAAAGCATAATGC
CCAGGGGCAGGGTAATGGGTTGCGATATGGGCTAAGCAGCATGC
AAGGCTGGCGTGTGAAATGGAGGATGCACATACGGCTGTGATC
GGTTTGCCAAGTGGACTTGAATCGTGGTCATTCTTTGCTGTGTA
TGATGGGCATGCTGGTTCTCAGGTTGCCAAATACTGCTGTGAGC
ATTTGTTAGATCACATCACCAATAACCAGGATTTTAAAGGGTCT
GCAGGAGCACCTTCTGTGGAAAATGTAAAGAATGGAATCAGAAC
AGGTTTTCTGGAGATTGATGAACACATGAGAGTTATGTCAGAGA
AGAAACATGGTGCAGATAGAAGTGGGTCAACAGCTGTAGGTGTC
TTAATTTCTCCCCAACATACTTATTTTATTAACTGTGGAGACTC
AAGAGGTTTACTTTGTAGGAACAGGAAAGTTCATTTCTTCACAC
AAGATCACAAACCAAGTAATCCGCTGGAGAAAGAACGAATTCAG
AATGCAGGTGGCTCTGTAATGATTCAGCGTGTGAATGGCTCTCT
GGCTGTATCGAGGGCCCTTGGGGATTTTGATTACAAATGTGTCC
ATGGAAAAGGTCCTACTGAGCAGCTTGTCTCACCAGAGCCTGAA
GTCCATGATATTGAAAGATCTGAAGAAGATGATCAGTTCATTAT
CCTTGCATGTGATGGTATCTGGGATGTTATGGGAAATGAAGAGC
TCTGTGATTTTGTAAGATCCAGACTTGAAGTCACTGATGACCTT
GAGAAAGTTTGCAATGAAGTAGTCGACACCTGTTTGTATAAGGG
AAGTCGAGACAACATGAGTGTGATTTTGTATCTGTTTTCCAAATG
CACCCAAAGTATCGCCAGAAGCAGTGAAGAAGGAGGCAGAGTTG
GACAAGTACCTGGAATGCAGAGTAGAAGAAATCATAAAGAAGCA
GGGGGAAGGCGTCCCCGACTTAGTCCATGTGATGCGCACATTAG
CGAGTGAGAACATCCCCAGCCTCCCACCAGGGGGTGAATTGGCA
AGCAAGAGGAATGTTATTGAAGCCGTTTACAATAGACTGAATCC
TTACAAAAATGACGACACTGACTCTACATCAACAGATGATATGT
GGtaa