Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of 14-3-3 gamma [1 - 247]

Protein description:- 14-3-3 gamma [1 - 247]

Clone number:- DU 8262

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 10 mg/L

Calculated molecular mass:-

Monoisotopic 31, 826.47 daltons
Average Mass 31, 846.49 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.34

Purity:- >85 %

Enzyme storage buffer:-

25 mM Hepes pH 7.5, 50 % glycerol, 1 mM DTT, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- –20 °C

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CLONE DATA SHEET

14-3-3 gamma [1 - 247]

Protein 14-3-3 gamma [1 - 247]

Clone number DU 8262

Species Human

Accession no BC020963

Tags N-terminal His(6)

Bacterially expressed protein

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS**MVDREQLVQKAR LAEQAERYDDMAAAMKNVTELNEPLSNEERNLLSVAYKNVVGARRS SWRVISSIEQKTSADGNEKKIEMVRAYREKIEKELEAVCQDVLSLL DNYLIKNCSETQYESKVFYLKMKGDYYRYLAEVATGEKRATVVESS EKAYSEAHEISKEHMQPTHPIRLGLALNYSVFYYEIQNAPEQACHL AKTAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQDDDG GEGNN**

Native sequence Amino acids M1 – N247 (end) of human 14-3-3 gamma. Residue M35 of the fusion protein is equivalent to M1 of the native protein. The His(6) tag is located at residues 5 – 10.

Protease cleavage Thrombin (LVPRGS) between residues 14 - 19

Cloning sites *Bam*H1 and *Not*1 of pET 28a

Nucleotide sequence of insert

ggatccATGGTGGACCGCGAGCAACTGGTGCAGAAAGCCCGGCTGG
CCGAGCAGGCGGAGCGCTACGACGACATGGCCGCGGCCATGAAGAA
CGTGACAGAGCTGAATGAGCCACTGTCGAATGAGGAACGAAACCTT
CTGTCTGTGGCCTACAAGAACGTTGTGGGGGCACGCCGCTCTTCCT
GGAGGGTCATCAGTAGCATTGAGCAGAAGACATCTGCAGACGGCAA
TGAGAAGAAGATTGAGATGGTCCGTGCGTACCGGGAGAAGATAGAG
AAGGAGTTGGAGGCTGTGTGCCAGGATGTGCTGAGCCTGCTGGATA
ACTACCTGATCAAGAATTGCAGCGAGACCCAGTACGAGAGCAAAGT
GTTCTACCTGAAGATGAAAGGGGACTACTACCGCTACCTGGCTGAA
GTGGCCACCGGAGAGAAAAGGGCGACGGTGGTGGAGTCCTCTGAGA
AGGCCTACAGCGAAGCCCACGAGATCAGCAAAGAGCACATGCAGCC
CACCCACCCCATCCGATTAGGCCTGGCTCTTAACTACTCCGTCTTC
TACTATGAGATCCAGAACGCCCCAGAGCAAGCGTGCCACTTGGCCA
AGACCGCGTTCGACGACGCCATCGCCGAGCTTGACACCCTCAACGA

GGACTCCTACAAGGACTCCACGCTCATCATGCAGCTCCTCCGCGAC
AACCTCACGCTCTGGACGAGCGACCAGCAGGACGACGATGGCGGCG
AAGGCAACAATtaagcggccgc