Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of 14-3-3 eta [1 - 246]

Protein description:-   14-3-3 eta [1 - 246]

Clone number:-   DU 8263

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, 742.58 daltons
Average Mass   31, 762.62 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:-   5.3

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 eta [1 - 246]

Protein   14-3-3 eta [1 - 246]

Clone number   DU 8263

Species   Human

Accession no   NM_003405

Tags   N-terminal His(6)

Bacterially expressed protein

MGSSHHHHHHSSG<u>LVPRGS</u>HMASMTGGQQMGRGS**MGDREQLLQRAR LAEQAERYDDMASAMKAVTELNEPLSNEDRNLLSVAYKNVVGARRS SWRVISSIEQKTMADGNEKKLEKVKAYREKIEKELETVCNDVLSLL DKFLIKNCNDFQYESKVFYLKMKGDYYRYLAEVASGEKKNSVVEAS EAAYKEAFEISKEQMQPTHPIRLGLALNFSVFYYEIQNAPEQACLL AKQAFDDAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDQQDEEA GEGN**

Native sequence

Amino acids M1 – N246 (end) of human 14-3-3 eta.
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 (<u>LVPRGS</u>) between residues 14 - 19

Cloning sites

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

Nucleotide sequence of insert

ggatccATGGGGGACCGGGAGCAGCTGCTGCAGCGGGCGCGGCTGG CCGAGCAGGCGGAGCGCTACGACGACATGGCCTCCGCTATGAAGGC GGTGACAGAGCTGAATGAACCTCTCTCCAATGAAGATCGAAATCTC CTCTCTGTGGCCTACAAGAATGTGGTTGGTGCCAGGCGATCTTCCT GGAGGGTCATTAGCAGCATTGAGCAGAAAACCATGGCTGATGGAAA CGAAAAGAAATTGGAGAAAGTTAAAGCTTACCGGGAGAAGATTGAG AAGGAGCTGGAGACAGTTTGCAATGATGTCCTGTCTCTGCTTGACA AGTTCCTGATCAAGAACTGCAATGATTTCCAGTATGAGAGCAAGGT GTTTTACCTGAAAATGAAGGGTGATTACTACCGCTACTTAGCAGAG GTCGCTTCTGGGGAGAAGAAAAACAGTGTGGTCGAAGCTTCTGAAG CTGCCTACAAGGAAGCCTTTGAAATCAGCAAAGAGCAGATGCAACC CACGCATCCCATCCGGCTGGGCCTGGCCCTCAACTTCTCCGTGTTC TACTATGAGATCCAGAATGCACCTGAGCAAGCCTGCCTCTTAGCCA AACAAGCCTTCGATGATGCCATAGCTGAGCTGGACACACTAAACGA

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GGATTCCTATAAGGACTCCACGCTGATCATGCAGTTGCTGCGAGAC
AACCTCACCCTCTGGACGAGCGACCAGCAGGATGAAGAAGCAGGAG
AAGGCAACtgagcggccgc
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