Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of 14-3-3 theta [1 - 245]

Protein description:- 14-3-3 theta [1 - 245]

Clone number:- DU 8265

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, 288.32 daltons
Average Mass	31, 308.15 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.2

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 theta [1 - 245]

Protein	14-3-3 theta [1 - 245]
Clone number	DU 8265
Species	Human
Accession no	NM_006826
Tags	N-terminal His(6)
Bacterially expressed protein	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS**MEKTELIQKAKL** **AEQAERYDDMATCMKAVTEQGAELSNEERNLLSVAYKNVVGGRRSA** **WRVISSIEQKTDTSDKKLQLIKDYREKVESELRSICTTVLELLDKY** **LIANATNPESKVFYLKMKGDYFRYLAEVACGDDRKQTIDNSQGAYQ** **EAFDISKKEMQPTHPIRLGLALNFSVFYYEILNNPELACTLAKTAF** **DEAIAELDTLNEDSYKDSTLIMQLLRDNLTLWTSDSAGEECDAAEG** **AEN**
Native sequence	Amino acids M1 – N245 (end) of human 14-3-3 theta. 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	ggatccATGGAGAAGACTGAGCTGATCCAGAAGGCCAAGCTGGCCG AGCAGGCCGAGCGCTACGACGACATGGCCACCTGCATGAAGGCAGT GACCGAGCAGGGCGCCGAGCTGTCCAACGAGGAGCGCAACCTGCTC TCCGTGGCCTACAAGAACGTGGTCGGGGGCCGCAGGTCCGCCTGGA GGGTCATCTCTAGCATCGAGCAGAAGACCGACACCTCCGACAAGAA GTTGCAGCTGATTAAGGACTATCGGGAGAAAGTGGAGTCCGAGCTG AGATCCATCTGCACCACGGTGCTGGAATTGTTGGATAAATATTTAA TAGCCAATGCAACTAATCCAGAGAGTAAGGTCTTCTATCTGAAAAT GAAGGGTGATTACTTCCGGTACCTTGCTGAAGTTGCGTGTGGTGAT GATCGAAAACAAACGATAGATAATTCCCAAGGAGCTTACCAAGAGG CATTTGATATAAGCAAGAAAGAGATGCAACCCACACACCCAATCCG CCTGGGGCTTGCTCTTAACTTTTCTGTATTTTACTATGAGATTCTT AATAACCCAGAGCTTGCCTGCACGCTGGCTAAAACGGCTTTTGATG AGGCCATTGCTGAACTTGATACACTGAATGAAGACTCATACAAAGA

CAGCACCCTCATCATGCAGTTGCTTAGAGACAACCTAACACTTTGG
ACATCAGACAGTGCAGGAGAAGAATGTGATGCGGCAGAAGGGGCTG
AAAACtaagcggccgc