

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of 14-3-3 sigma [1 - 248]

Protein description:- 14-3-3 sigma [1 - 248]

Clone number:- DU 8264

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

Purification method:- Ni²⁺-NTA agarose

Expression level:- 10 mg/L

Calculated molecular mass:-

Monoisotopic 31, 298.25 daltons
Average Mass 31, 317.96 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.11

Purity:- >85 %

Enzyme storage buffer:-

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

Storage temperature:- -20 °C

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CLONE DATA SHEET

14-3-3 sigma [1 - 248]

<u>Protein</u>	14-3-3 sigma [1 - 248]
<u>Clone number</u>	DU 8264
<u>Species</u>	Human
<u>Accession no</u>	NM_006142
<u>Tags</u>	N-terminal His(6)
<u>Bacterially expressed protein</u>	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMERASLIQKAKL AEQAERYEDMAAFMKGAVEKGEELSCEERNLLSVAYKNVVGQRAA WRVLSSEIEQKSNEEGSEEKGPVREYREKRVETELQGVCDTVLGLLD SHLIKEAGDAESRVFYLKMKGDYRYLAEVATGDDKKRIIDSARSA YQEAMDISKKEMPPNPIRLGLALNFSVFHYEIANSPPEAISLAKT TFDEAMADLHTLSEDSYKDSTLIMQLLRDNLTLWTADNAGEEGGEA PQEPQS
<u>Native sequence</u>	Amino acids M1 – S248 (end) of human 14-3-3 sigma. Residue M35 of the fusion protein is equivalent to M1 of the native protein. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	Thrombin (<u>LVPRGS</u>) between residues 14 - 19
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 of pET 28a
<u>Nucleotide sequence of insert</u>	ggatccATGGAGAGAGCCAGTCTGATCCAGAAGGCCAAGCTGGCAG AGCAGGCCGAACGCTATGAGGACATGGCAGCCTTCATGAAAGGCGC CGTGGAGAAGGGCGAGGAGCTCTCCTGCGAAGAGCGAAACCTGCTC TCAGTAGCCTATAAGAACGTGGTGGGCGGCCAGAGGGCTGCCTGGA GGGTGCTGTCCAGTATTGAGCAGAAAAGCAACGAGGAGGGCTCGGA GGAGAAGGGGCCCGAGGTGCGTGAGTACCGGGAGAAGGTGGAGACT GAGCTCCAGGGCGTGTGCGACACCGTGCTGGGCCCTGCTGGACAGCC ACCTCATCAAGGAGGCCGGGGACGCCGAGAGCCGGGTCTTCTACCT GAAGATGAAGGGTGACTACTACCGCTACCTGGCCGAGGTGGCCACC GGTGACGACAAGAAGCGCATCATTGACTCAGCCCGGTGAGCCTACC AGGAGGCCATGGACATCAGCAAGAAGGAGATGCCGCCACCAACCC CATCCGCCTGGGCCTGGCCCTGAACTTTTCCGTCTTCCACTACGAG ATCGCCAACAGCCCCGAGGAGGCCATCTCTCTGGCCAAGACCACTT TCGACGAGGCCATGGCTGATCTGCACACCCTCAGCGAGGACTCCTA

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CAAAGACAGCACCCATCATGCAGCTGCTGCGAGACAACCTGACA
CTGTGGACGGCCGACAACGCCGGGAAGAGGGGGCGAGGCTCCCC
AGGAGCCCCAGAGctgagcggccgc