

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Ubc H3 [2 - 236]

Enzyme description:- Ubc H3 [2 – 236]

Clone number:- DU 4317

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

Purification method:- Ni²⁺-NTA agarose

Expression level:- 30 mg/L

Calculated molecular mass:-

Monoisotopic 30, 130.56 daltons
Average Mass 30, 149.47 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.80

Purity:- 90 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Ubiquitin assay

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Clone Data Sheet

Ubc H3 [2 - 236]

| | |
|---|---|
| <u>Protein</u> | Ubc H3 [2 - 236] |
| <u>Clone Number</u> | DU 4317 |
| <u>Species</u> | Human |
| <u>Accession number</u> | NM_004359 |
| <u>Tags</u> | N-terminal His(6) |
| <u>Bacterially expressed protein</u> | MGSSHHHHHSSGLVPRGSHMASMTGGQQMGRGSARPLVPSSQKALLE LKGLQEEPVEGFRVTLVDEGDLYNWEVAIFGPPNTYYEGGYFKARLKFP IDYPYSPPAFRFLTKMHPNIYETGDVCISILHPPVDDPQSGELPSERW NPTQNVRTILLSVISLLNEPNTFSPANVDASVMYRKWKESKGDREYTD IIRKQVLGTKVDAERDGVKVPPTTAEYCVKTKAPAPDEGSDFYDDYYE DGEVEEEADSCFGDEDDSGTEES |
| <u>Native sequence</u> | Amino acids A2 – S269 (end) of Ubc H3. Residue A35 of the fusion protein is equivalent to A2 of the native enzyme. The His(6) tag is located at residues 5 – 10. |
| <u>Protease cleavage</u> | Thrombin (<u>LVPRGS</u>) residues 14 - 19 |
| <u>Cloning sites</u> | <i>Bam</i> H1 and <i>Not</i> 1 site of pET 28a (+) |

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**Nucleotide
Sequence**

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGC
GCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTGCGGG
ATCCGCTCGGCCGCTAGTGCCCAGCTCGCAGAAGGCGCTGCTGCTGGAG
CTCAAGGGGCTGCAGGAAGAGCCGGTCGAGGGATTCCGCGTGACACTGG
TGGACGAGGGCGATCTATACAACTGGGAGGTGGCCATCTTCGGGCCCC
CAACACCTACTACGAGGGCGGCTACTTCAAGGCGCGCCTCAAGTTCCCC
ATCGACTACCCATACTCTCCACCAGCCTTTCGGTTCCTGACCAAGATGT
GGCACCCCTAACATCTACGAGACGGGGGACGTGTGTATCTCCATCCTCCA
CCCGCCGGTGGACGACCCCCAGAGCGGGGAGCTGCCCTCAGAGAGGTGG
AACCCACGCAGAACGTCAGGACCATTCTCCTGAGTGTGATCTCCCTCC
TGAACGAGCCCAACACCTTCTCGCCCGCAAACGTGGACGCCTCCGTGAT
GTACAGGAAGTGGAAGAGAGCAAGGGGAAGGATCGGGAGTACACAGAC
ATCATCCGGAAGCAGGTCCTGGGGACCAAGGTGGACGCGGAGCGTGACG
GCGTGAAGGTGCCACCACGCTGGCCGAGTACTGCGTGAAGACCAAGGC
GCCGGCGCCCGACGAGGGCTCAGACCTTCTACGACGACTACTACGAG
GACGGCGAGGTGGAGGAGGAGGCCGACAGCTGCTTCGGGGACGATGAGG
ATGACTCTGGCACGGAGGAGTCCtgagcggccgcc