Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of His-Ubiquitin [2 - 76]

Enzyme description:- His-Ubiquitin [2 - 76]

Clone number:- DU 3351

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

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

Expression level:- 0.5 mg/L

Calculated molecular mass:-

Monoisotopic 11,970.13 daltons
Average Mass 11,977.55 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.16

Purity:- 85 %

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

Ubiquitin [2 - 76]

Protein	Ubiquitin [2 - 76]
Clone Number	DU 3551
Species	Human
Accession number	P62988
Tags	N-terminal His(6)
Bacterially expressed protein	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS**QIFVKTLTGKTITLE VEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKES TLHLVLRLRGG**
Native sequence	Amino acids Q2 – G76 (end) of human Ubiquitin. Residue Q35 of the fusion protein is equilivalent to Q2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	None
Cloning sites	*Bam*H1 and *Not*1 site of pET28a
Nucleotide Sequence	ggatccCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTCG AAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATCCAAGA CAAGGAAGGCATCCCTCCTGACCAGCAGAGGTTGATCTTTGCTGGGAAA CAGCTGGAAGATGGACGCACCCTGTCTGACTACAACATCCAGAAAGAGT CCACCCTGCACCTGGTCCTCCGTCTCAGAGGTGGGtgataactcgag