Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UCHL1

Enzyme description:- His-UCHL1

Clone number:- DU15693

Source:- BL21 Recombinant

Tag:- N-terminal His$_6$-tag

Purification method:- Ni^{++}-Sepharose

Expression level:- 6 mg/L

Calculated molecular mass:-

Monoisotopic	27251 Da
Average Mass	27267 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.92

Purity:- 95%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80ºC

Assay:-

Ub-Rho110-Gly cleavage assay monitored by Ex/Em 485/535 nm

Assay buffer:-

40 mM Tris pH 7.5, 100 mM NaCl, 5 mM DTT, 0.01% Triton X-100, 0.005% Ovalbumin, 0.5 μM Ub-Rho110-Gly

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Clone Data Sheet

His-UCHL1

Protein	His-UCHL1
Synonyms	
Clone Number	DU15693
Species	Human
Accession Number	Protein: P09936 DNA: AAH00332.1
Tags	N-terminal His_6-tag
Amino acid sequence of expressed protein	MGSSHHHHHHSSGLEVLFQGPGS**MQLKPMEINPEMLNKVLSRLGVAGQWRFVDVLGLEEESLGSVPAPACALLLLFPLTAQHENFRKKQIEELKGQEVSPKVYFMKQTIGNSCGTIGLIHAVANNQDKLGFEDGSVLKQFLSETEKMSPEDRAKCFEKNEAIQAAHDAVAQEGQCRVDDKVNFHFILFNNVDGHLYELDGRMPFPVNHGASSEDTLLKDAAKVCREFTEREQGEVRFSAVALCKAA**
Native sequence	in bold
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1
DNA sequence of insert	GGATCCATGCAGCTCAAGCCGATGGAGATCAACCCCGAGATGCTGAACAAAGTGCTGTCCCGGCTGGGGGTCGCCGGCCAGTGGCGCTTCGTGGACGTGCTGGGGCTGGAAGAGGAGTCTCTGGGCTCGGTGCCAGCGCCTGCCTGCGCGCTGCTGCTGCTGTTTCCCCTCACGGCCCAGCATGAGAACTTCAGGAAAAAGCAGATTGAAGAGCTGAAGGGACAAGAAGTTAGTCCTAAAGTGTACTTCATGAAGCAGACCATTGGGAATTCCTGTGGCACAATCGGACTTATTCACGCAGTGGCCAATAATCAAGACAAACTGGGATTTGAGGATGGATCAGTTCTGAAACAGTTTCTTTCTGAAACAGAGAAAATGTCCCCTGAAGACAGAGCAAAATGCTTTGAAAAGAATGAGGCCATACAGGCAGCCCATGATGCCGTGGCACAGGAAGGCCAATGTCGGGTAGATGACAAGGTGAATTTCCATTTTATTCTGTTTAACAACGTGGATGGCCACCTCTATGAACTTGATGGACGAATGCCTTTTCCGGTGAACCATGGCGCCAGTTCAGAGGACACCCTGCTGAAGGACGCTGCCAAGGTCTGCAGAGAATTCACCGAGCGTGAGCAAGGAGAAGTCCGCTTCTCTGCCGTGGCTCTCTGCAAGGCAGCCTAAGCGGCCGC