Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-TEV-Halo-TUBE

<u>Enzyme description:-</u> Ubiquilin UBA domain x 4 = TUBE

<u>Clone number:-</u> DU23799

<u>Source:-</u> human recombinant

<u>Tag:-</u> N-terminal His-TEV

<u>Purification method:-</u> Ni^{++}-NTA-Sepharose

<u>Expression system:-</u> E.coli

<u>Calculated molecular mass:-</u>

Monoisotopic	60816
Average Mass	60852

[cysteines reduced, methionines have not been oxidised]

<u>Theoretical pI:-</u> 4.88

<u>Purity:-</u> 90%

<u>Enzyme storage buffer:-</u>

50 mM HEPES pH 7.5, 150mM NaCl, 1mM DTT

<u>Storage temperature:-</u> -80ºC

<u>Assay:-</u>

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Clone Data Sheet

Protein name His-Halo-TUBE

Protein	**His-Halo-TUBE**
Synonyms	tetramer of Ubiquilin-1 UBA domain
Clone Number	DU23799
Species	human
Accession Number	Protein: Q9UMX0
Tags	N-terminal His-TEV-
Aminoacid sequence of the expressed protein	MGSSHHHHHHENLYFQ*MAEIGTGFPFDPHYVEVLGERMHYVDVGPRD* *GTPVLFLHGNPTSSYVWRNIIPHVAPTHRCIAPDLIGMGKSDKPDLG* *YFFDDHVRFMDAFIEALGLEEVVLVIHDWGSALGFHWAKRNPERVKG* *IAFMEFIRPIPTWDEWPEFARETFQAFRTTDVGRKLIIDQNVFIEGT* *LPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIV* *ALVEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVD* *IGPGLNLLQEDNPDLIGSEIARWLSTLEIS*GGSGGG**VNPQLQNPEVR** **FQQQLEQLSAMGFLNREANLQALIATGGDINAAIERLLGSQPSGGGG** **SGGGVNPQLQNPEVRFQQQLEQLSAMGFLNREANLQALIATGGDINA** **AIERLLGSQPSGGGGSGGGVNPQLQNPEVRFQQQLEQLSAMGFLNRE** **ANLQALIATGGDINAAIERLLGSQPSGGGGSGGGVNPQLQNPEVRFQ** **QQLEQLSAMGFLNREANLQALIATGGDINAAIERLLGSQPS**
Native sequence	Ubiquilin1 is a 597 residue protein. The UBA domain spans residues 536 – 589. This protein (DU23799) is a tetramer of the UBA domain, referred to as Tandem-Ubiquitin-Binding-Entity = **TUBE in bold,** *Halo-tag in italic*
Protease cleavage	TEV protease underlined
Cloning sites	BglII/BamHI into BamHI

DNA sequence of the expression cassette

ATGGGCAGCAGCCATCATCATCATCATCACGAAAACCTGTATTTTCAGatggc
agaaatcggtactggctttccattcgacccccattatgtggaagtcctgggcg
agcgcatgcactacgtcgatgttggtccgcgcgatggcacccctgtgctgttc
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acaaaccagacctgggttatttcttcgacgaccacgtccgcttcatggatgcc
ttcatcgaagccctgggtctggaagaggtcgtcctggtcattcacgactgggg
ctccgctctgggtttccactgggccaagcgcaatccagagcgcgtcaaaggta
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GTAAATCCTCAGCTACAGAATCCAGAAGTCAGATTTCAGCAACAACTGGAACA
ACTCAGTGCAATGGGATTTTTGAACCGTGAAGCAAACTTGCAAGCTCTAATAG
CAACAGGAGGTGATATCAATGCAGCTATTGAAAGGTTACTGGGCTCCCAGCCA
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GCAACAGGAGGTGATATCAATGCAGCTATTGAAAGGTTACTGGGCTCCCAGCC
ATCAGGAGGTGGAGGATCTGGAGGTGGAGTAAATCCTCAGCTACAGAATCCAG
AAGTCAGATTTCAGCAACAACTGGAACAACTCAGTGCAATGGGATTTTTGAAC
CGTGAAGCAAACTTGCAAGCTCTAATAGCAACAGGAGGTGATATCAATGCAGC
TATTGAAAGGTTACTGGGCTCCCAGCCATCATAG