

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of UFC1 [1 – 167]**

**Enzyme description:-** UFC1 [1 – 167]

**Clone number:-** DU 53290

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal His(6)

**Purification method:-** Cobalt Agarose

**Calculated molecular mass:-**

Monoisotopic 21, 829.06 daltons

Average Mass 21, 842.98 daltons

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.79

**Purity:-** >80 %

**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, 1 mM benzamidine, 0.2 mM PMSF

**Storage temperature:-** -70 °C

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**Clone Data Sheet**

**UFC1 [1 – 167]**

<b><u>Protein</u></b>	UFC1 [1 – 167]
<b><u>Clone number</u></b>	DU 53290
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_016406.3
<b><u>Tags</u></b>	N-terminal His6
<b><u>Bacterially expressed protein</u></b>	<b>MGSSHHHHHSSGLEVLFGPGSMAD<del>E</del>ATRRVVSEIPVLKTNAGPRDRE LWVQRLKEEYQSLIRYVENNKNADNDWFRLESNKEGTRWFGKCWYIHDL LKYEFDIEFDIPITCPTTAPEIAVPELDGKTAKMYRGGKICLTDHFKPL WARNVPKFGLAHLMALGLGPWLAVEIPDLIQKGVIOHKEKCNQ</b>
<b><u>Native sequence</u></b>	Amino acids M1 – Q167 (end) of human UFC1. Residue M24 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFGGP</u> ) residues 14 - 21
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pET15b His6

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**Nucleotide**  
**Sequence of insert**

ggatccATGGCGGATGAAGCCACGCGACGTGTTGTGTCTGAGATCCCGG  
TGCTGAAGACTAACGCCGGACCCCGAGATCGTGAGTTGTGGGTGCAGCG  
ACTGAAGGAGGAATATCAGTCCCTTATCCGGTATGTGGAGAACAACAAG  
AATGCTGACAACGATTGGTTCCGACTGGAGTCCAACAAGGAAGGAAGTCTC  
GGTGGTTTGGAAAATGCTGGTATATCCATGACCTCCTGAAATATGAGTT  
TGACATCGAGTTTGACATTCCTATCACATGTCCTACTACTGCCCCAGAA  
ATTGCAGTTCCTGAGCTGGATGGAAAGACAGCAAAGATGTACAGGGGTG  
GCAAATATGCCTGACGGATCATTTCAAACCTTTGTGGGCCAGGAATGT  
GCCCAAATTTGGACTAGCTCATCTCATGGCTCTGGGGCTGGGTCCATGG  
CTGGCAGTGGAAATCCCTGATCTGATTCAGAAGGGCGTCATCCAACACA  
AAGAGAAATGCAACCAAtgagcggccgc