

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of Ub trimer (linear)**

<b><u>Enzyme description:-</u></b>	Ubiquitin-Ubiquitin-Ubiquitin
<b><u>Clone number:-</u></b>	DU20730
<b><u>Source:-</u></b>	BL21 recombinant
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH-Sepharose, protease treatment Depletion over Q-Sepharose, Source 15 S
<b><u>Expression level:-</u></b>	1mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	26182 Da
Average Mass	26197 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	7.7
<b><u>Purity:-</u></b>	90%
<b><u>Enzyme storage buffer:-</u></b>	
50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<b><u>Storage temperature:-</u></b>	-80°C
<b><u>Assay:-</u></b>	

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**Clone Data Sheet**

**Ubiquitin-trimer (linear)**

<b><u>Protein</u></b>	Ubiquitin trimer (linear)
<b><u>Synonyms</u></b>	Ubiquitin-60S ribosomal protein L40; AltName: CEP52; Ubiquitin A-52 residue ribosomal protein fusion product 1
<b><u>Clone Number</u></b>	DU20730
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: P62987
<b><u>Tags</u></b>	cleaved from GST
Aminoacid sequence of the expressed protein	<u>G</u> PLGSAGM <u>Q</u> IFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQ QRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGGM <u>Q</u> IFVKTLTGK TITLEVEPSDTIENVKAKIQDKEGIPPDQORLIFAGKQLEDGRTLSD YNIQKESTLHLVLRRLRGGM <u>Q</u> IFVKTLTGKTITLEVEPSDTIENVKAK IQDKEGIPPDQORLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGG
Native sequence	
Protease cleavage	Prescission Protease
Cloning sites	NaeI / SfoI

DNA sequence of  
expression cassette

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACC  
CACTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATT  
TGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAA  
TTGGGTTTGGAGTTTCCCAATCTTCTTATTATATTGATGGTGATGT  
TAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGC  
ACAACATGTTGGGTGGTGTCCAAAAGAGCGTGCAGAGATTTCAATG  
CTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTCGAGAATTGC  
ATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGC  
TACCTGAAATGCTGAAAATGTTTCGAAGATCGTTTATGTCATAAAACA  
TATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGA  
CGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCTGGATGCGT  
TCCCAAATTAGTTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAA  
ATTGATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCA  
GGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGG  
ATCTGGAAGTTCTGTTCCAGGGGCCCTGGGATCC**GCCGGCATGCAG**  
**ATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTCGAAGTGGA**  
**GCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATCCAAGACAAGG**  
**AAGGCATCCCTCCTGACCAGCAGAGGTTGATCTTTGCTGGGAAACAG**  
**CTGGAAGATGGACGCACCCTGTCTGACTACAACATCCAGAAAGAGTC**  
**CACCCTGCACCTGGTCCTCCGTCTCAGAGGCGGCATGCAGATCTTCG**  
**TGAAGACCCTGACTGGTAAGACCATCACTCTCGAAGTGGAGCCGAGT**  
**GACACCATTGAGAATGTCAAGGCAAAGATCCAAGACAAGGAAGGCAT**  
**CCCTCCTGACCAGCAGAGGTTGATCTTTGCTGGGAAACAGCTGGAAG**  
**ATGGACGCACCCTGTCTGACTACAACATCCAGAAAGAGTCCACCCTG**  
**CACCTGGTCCTCCGTCTCAGAGGCGGCATGCAGATCTTCGTGAAGAC**  
**CCTGACTGGTAAGACCATCACTCTCGAAGTGGAGCCGAGTGACACCA**  
**TTGAGAAATGTCAAGGCAAAGATCCAAGACAAGGAAGGCATCCCTCCT**  
**GACCAGCAGAGGTTGATCTTTGCTGGGAAACAGCTGGAAGATGGACG**  
**CACCCTGTCTGACTACAACATCCAGAAAGAGTCCACCCTGCACCTGG**  
**TCCTCCGTCTCAGAGGCGGCTGATAAGCGGCCGC**