

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of TRAF6**

<b><u>Enzyme description:-</u></b>	TRAF6 2-522 (end)
<b><u>Clone number:-</u></b>	DU43249
<b><u>Source:-</u></b>	insect recombinant
<b><u>Tag:-</u></b>	cleaved from Dac-TEV-
<b><u>Purification method:-</u></b>	Ampicillin-Sepharose
<b><u>Expression level:-</u></b>	0.5mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	59576 Da
Average Mass	59614Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	6.32
<b><u>Purity:-</u></b>	50%
<b><u>Enzyme storage buffer:-</u></b>	
50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM TCEP	
<b><u>Storage temperature:-</u></b>	-80°C

#### **Assay:-**

Formation of unanchored poly – ubiquitin chains with ubiquitin, UBE1, UBE2N/UBE2V1 in the presence of Mg-ATP.

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**Clone Data Sheet**

**TRAF6**

<b><u>Protein</u></b>	TRAF6 2-522
<b><u>Synonyms</u></b>	RNF85
<b><u>Clone Number</u></b>	DU43249
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: Q9Y4K3
<b><u>Tags</u></b>	cleaved from N-terminal Dac-
Aminoacid sequence of the expressed protein	<b>GGSLNCENSCGFSQSESDCCVAMASSCSAVTKDDSVGGTASTGNLSSSFME EIQGYDVEFDPPLESKYECPICLMALREAVQTPCGHRFCKACIIKSI RDAGH KCPVDNEILLENQLFPDNFAKREILSLMVKCPNEGCLHKMELRHLEDHQAH EFALMDCPQCQRPFQKFHINIHILKDCPRRQVSCDNCAASMAFEDKEIHDQ CPLANVICEYCNTILIREQMPNHYDLDCPTAPIPCTFSTFGCHEKMQRNHLA RHLQENTQSHMRMLAQAVHLSVIPDSGYISEVRNFQETIHQLEGRLVRQDH QIRELTAKMETQSMYVSELKRTIRTLEDKVAEIEAQQCNGIYIWKIGNFGMH LKCQEEKPVVIHSPGFYTGKPGYKLCMRLHLQLPTAQRANYISLHVHTMQ GEYDShLPWPFQGTIRLTILDQSEAPVRQNHHEIMDAKPELLAFQRPTIPRN PKGFGYVTFMHLEALRQRTFIKDDTLLVRCVSTRFDMGSLRREGFQPRSTD AGV</b>
Native sequence	in bold
Protease site	TEV-protease
Cloning sites	BamH1/NotI

**DNA sequence of  
the insert**

GGATCCAGTCTGCTAAACTGTGAAAACAGCTGTGGATTTCAGCCAGTCTGA  
AAGTGACTGCTGTGTGGCCATGGCCAGCTCCTGTAGCGCTGTAACAAAAG  
ATGATAGTGTGGGTGGAAGTCCAGCACGGGGAACCTCTCCAGCTCATT  
ATGGAGGAGATCCAGGGATATGATGTAGAGTTTGACCCACCCCTGGAAAG  
CAAGTATGAATGCCCCATCTGCTTGATGGCATTACGAGAAGCAGTGCAAA  
CGCCATGCGGCCATAGGTTCTGCAAAGCCTGCATCATAAAAATCAATAAGG  
GATGCAGGTCACAAATGTCCAGTTGACAATGAAATACTGCTGGAAAATCA  
ACTATTTCCAGACAATTTTGCAAAACGTGAGATTCTTTCTCTGATGGTGA  
AATGTCCAAATGAAGGTTGTTTGCACAAGATGGAAGTGGAGACATCTTGAG  
GATCATCAAGCACATTGTGAGTTTGCTCTTATGGATTGTCCCAATGCCA  
GCGTCCCTTCCAAAATTCATATTAATATTCACATTCTGAAGGATTGTC  
CAAGGAGACAGGTTTCTTGTGACAAGTGTGCTGCATCAATGGCATTGAA  
GATAAAGAGATCCATGACCAGAAGTGTCTTTGGCAAATGTCATCTGTGA  
ATACTGCAATACTATACTCATCAGAGAACAGATGCCTAATCATTATGATC  
TAGACTGCCCTACAGCCCCAATTCATGCACATTCAGTACTTTTGGTTGC  
CATGAAAAGATGCAGAGGAATCACTTGGCACGCCACCTACAAGAGAACAC  
CCAGTCACACATGAGAATGTTGGCCCAGGCTGTTTCATAGTTTGAGCGTTA  
TACCCGACTCTGGGTATATCTCAGAGGTCCGGAATTTCCAGGAACTATT  
CACCAGTTAGAGGGTCGCCTTGTAAGACAAGACCATCAAATCCGGGAGCT  
GACTGCTAAAATGGAAACTCAGAGTATGTATGTAAGTGAAGTCAAACGAA  
CCATTCGAACCCCTTGAGGACAAAAGTTGCTGAAATCGAAGCACAGCAGTGC  
AATGGAATTTATATTTGGAAGATTGGCAACTTTGGAATGCATTTGAAATG  
TCAAGAAGAGGAGAAAACCTGTTGTGATTTCATAGCCCTGGATTCTACTG  
GCAAACCCGGGTACAAACTGTGCATGCGCTTGCACCTTCAGTTACCGACT  
GCTCAGCGCTGTGCAAATATATATCCCTTTTGTCCACACAATGCAAGG  
AGAATATGACAGCCACCTCCCTTGGCCCTTCCAGGGTACAATACGCCTTA  
CAATTCTTGATCAGTCTGAAGCACCTGTAAGGCAAAACCACGAAGAGATA  
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GACAAAGAAGTTTCATTAAGGATGACACATTATTAGTGCCTGTGAGGTC  
TCCACCCGCTTTGACATGGGTAGCCTTCGGAGGGAGGGTTTTCAGCCACG  
AAGTACTGATGCAGGGGTATAGGCGGCCGCGC