

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of RNF8**

<b><u>Enzyme description:-</u></b>	GST-RNF8
<b><u>Clone number:-</u></b>	DU19751
<b><u>Source:-</u></b>	Recombinant
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH-Sepharose
<b><u>Expression level:-</u></b>	0.5mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	83500 Da
Average Mass	83632 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	6.70
<b><u>Purity:-</u></b>	80 %
<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**

**Protein name RNF8**

**Protein** GST-RNF8 1 - 485 (full length)

**Synonyms**

**Clone Number** DU19751

**Species** Human

**Accession Number** Protein: O76064

**Tags** N-terminal GST

Aminoacid sequence  
of the expressed  
protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL  
EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAELISMLEGAVL  
DIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTH  
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA  
WPLQGWQATFGGGDHPKSDLEVLFOGPLGSPEFPGRLERPL**MGEPEGFFV**  
**TGDRAGGRSWCLRRVGM SAGWLLLEDGCEVTVGRGFGV TYQLVSKICPLM**  
**ISRNHCVLKQNP EGQWTIMDNKSLNGVWLNRRARLEPLRVYSIHQDYIQL**  
**GVPLENKENAEY EYEVTEEDWETIYPCLSPKNDQMI EKNKELRTKRKFSL**  
**DELAGPGAEGPSNLKSKINKVSCESGQPVKSQ GKGEVASTPSDNLDPKLT**  
**ALEPSKTTGAPI YPGFPKVTEVHHEQKASNSSASQ RSLQMFV TMSRILR**  
**LKIQMQEKHEAVMNVKQTQKGN SSKVVQMEQELQDLQSQLCAEQ AQQQA**  
**RVEQLEKTFQEEEQHLOGLEIAQGEKDLKQQLAQALQEHWALMEELNRSK**  
**KDFEAI IQAKNKELEQTKEEKEKMQAQKEEVL SHMNDVLENELOCI ICSE**  
**YFIEAVTLNCAHSFCSYCIN EWMKRKIECPICRKDIKSKTYSLVLDNCIN**  
**KMVNNLSSEVKERRIVLIRERKAKRLF**

Native sequence in bold

Protease cleavage Precission site underlined

Cloning sites Not1

**DNA sequence of  
insert**

GCGGCCGCTCATGGGGGAGCCCGGCTTCTTCGTCACAGGAGACCGCGCCGG  
TGGCCGGAGCTGGTGCCTGCGGCGGGTGGGGATGAGCGCCGGGTGGCTGCT  
GCTGGAAGATGGGTGCGAGGTGACTGTAGGACGAGGATTTGGTGTACATA  
CCAAC TGGTATCAAAAATCTGCCCCCTGATGATTTCTCGAAACCACTGTGT  
TTTGAAGCAGAATCCTGAGGGCCAATGGACAATTATGGACAACAAGAGTCT  
AAATGGTGT TTTGGCTGAACAGAGCGCGTCTGGAACCTTTAAGGGTCTATTC  
CATTCATCAGGGAGACTACATCCAAC TGGAGTGCCTCTGGAAAATAAGGA  
GAATGCGGAGTATGAATATGAAGT TACTGAAGAAGACTGGGAGACAATATA  
TCCTTGTCTTTCCCAAAGAATGACCAAATGATAGAAAAAATAAGGAATT  
GAGAACTAAAAGGAAATTCAGTTTGGATGAATTAGCAGGTCCTGGAGCTGA  
AGGCCCTCAAATTTGAAATCCAAAATAAAATAAAGTGTCTTGTGAATCTGG  
TCAGCCAGTGAAATCACAGGGGAAAGGTGAAGTGGCCAGTACACCCTCTGA  
CAATTTGGATCCTAAGTTGACTGCCCTTGAGCCAAGTAAGACCACAGGGGC  
TCCATTTACCTGGCTTCCCAAAGTCACAGAGGTT CATCATGAGCAGAA  
AGCCTCAAAC TTT CAGCATCTCAGAGAAGCTTACAGATGTTTAAGGTGAC  
CATGTCCAGGATCTGAGGCTCAAATACAGATGCAGGAAAAACATGAAGC  
CGTTATGAATGTGAAAAAGCAGACCCAAAAGGGGAACTCAAAGAAAGTTGT  
GCAAATGGAGCAGGAACTTCAGGACTTACAGTCCCAGCTGTGTGCAGAGCA  
GGCTCAGCAGCAGGCAAGAGTGGAGCAACTAGAGAAGACTTTCAGGAAGA  
GGAACAGCATCTTCAGGGTTTGGAGATAGCCCAAGGAGAAAAGGACCTGAA  
GCAACAGCTGGCCAGGCTCTGCAGGAGCAT TGGGCTCTAATGGAAGAGCT  
AAATCGCAGCAAGAAGGACTTTGAAGCAATCATTCAAGCCAAGAACAAAAGA  
ATTAGAGCAGACCAAGGAAGAGAAGGAGAAGATGCAAGCACAGAAGGAAGA  
AGTTCTTAGCCACATGAATGATGTGCTAGAGAATGAGCTCCAATGTATTAT  
TTGTT CAGAATACTTCATTGAGGCTGTCACCTTGAAGTGTGCCACAGTTT  
CTGCTCCTACTGTATCAATGAATGGATGAAGCGGAAGATAGAATGCCCCAT  
TTGTCGGAAGGACATTAAGTCCAAAACATACTCTTTGGTCTGGACAATTG  
CATTAATAAGATGGTAAATAATCTGAGCTCAGAAGTGAAGAACGACGAAT  
TGTTCTCATTAGGGAACGAAAAGCAAAGAGATTGTTCTGAGCGGCCGC