

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of TNF alpha [77 – 233]**

**Enzyme description:-** TNF alpha [77 – 233]

**Clone number:-** DU 8654

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Calculated molecular mass:-**

Monoisotopic	44, 148.67 daltons [GST tag intact]
Monoisotopic	17, 753.24 daltons [cleaved of GST tag]
Average Mass	44, 176.89 daltons [GST tag intact]
Average Mass	17, 764.18 daltons [cleaved of GST tag]

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.04 (GST tag intact)  
7.02 (GST tag cleaved)

**Purity:-** >80 %

**Enzyme storage buffer:-**

Phosphate Buffered Saline

**Storage temperature:-** -70 °C

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**Clone Data Sheet**

**TNF alpha [77 – 233]**

<b><u>Protein</u></b>	TNF alpha [77 – 233]
<b><u>Clone number</u></b>	DU 8654
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	M10988.1
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGS<b>VRSSSRTPSDKPVA HVVANPQAEGLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVL FKGQGPCSTHVL</b>LTHTISRIAVSYQTKVNL<b>LSAIKSPCQRETPEGAEAK PWYEP</b>IYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGI<b>IAL</b></p>
<b><u>Bacterially expressed GST cleaved protein</u></b>	<p>GPLGS<b>VRSSSRTPSDKPV</b>AHVVANPQAEGLQWLNRRANALLANGVELR <b>DNQLVVPSEGLYLIYSQVLFKGQGPCSTHVL</b>LTHTISRIAVSYQTKVNL <b>LSAIKSPCQRETPEGAEAKPWYEP</b>IYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGI<b>IAL</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids V77 – L233 (end) of human PFKFB3. Residue V232 of the fusion protein is equivalent to V77 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<b><u>Protease cleavage</u></b>	PreScission ( <u>LEVLFQGP</u> ) residues 221 - 228
<b><u>Cloning sites</u></b>	<i>Bam</i> H1 site into pGEX6P-1

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**Nucleotide Sequence of Insert:**

ggatccGTCAGATCATCTTCTCGAACCCCGAGTGACAAGCCTGTAGCCCATGTTGTAGCAAACCCT  
CAAGCTGAGGGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCAATGGCGTGGAG  
CTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCCAGGTCCTCTTC  
AAGGGCCAAGGCTGCCCCTCCACCCATGTGCTCCTCACCCACACCATCAGCCGCATCGCCGTCTCC  
TACCAGACCAAGGTCAACCTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCAGAGGGG  
GCTGAGGCCAAGCCCTGGTATGAGCCCATCTATCTGGGAGGGTCTTCCAGCTGGAGAAGGGTGAC  
CGACTCAGCGCTGAGATCAATCGGCCCGACTATCTCGACTTTGCCGAGTCTGGGCAGGTCTACTTT  
GGGATCATTGCCCTGtgaggatcc

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