

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of TAU [1 – 441]**

<b><u>Enzyme description:-</u></b>	TAU [1 - 441]
<b><u>Clone number:-</u></b>	DU 32246
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	<i>E.coli</i>
<b><u>Tag:-</u></b>	N-terminal GST
<b><u>Purification method:-</u></b>	GSH Sepharose

#### **Calculated molecular mass:-**

Monoisotopic      72, 628.81 daltons  
Average Mass      72, 674.07 daltons  
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**                      6.68

**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**

#### **TAU [1 - 441]**

<b><u>Protein</u></b>	TAU [1 - 441]
<b><u>Clone number</u></b>	DU 32246
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_005910.5
<b><u>Tags</u></b>	N-terminal GST
<b><u>Bacterially expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWOATFGGGDHPPKSDLEVLFFQGPLGSMAEPRQEFVEMEDH <b>AGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEGSEEPGSET</b> <b>SDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTP</b> <b>SLEDEAAGHVTQARMVSKSKDGTGSDDKKAKGADGKTKIATPRGAAPPG</b> <b>QKGQANATRIPAKTPPAPKTPPSSGEPKSGDRSGYSSPGSPGTPGSRS</b> <b>RTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVMPDLKNVSKS</b> <b>IGSTENLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQI</b> <b>VYKPVDLSKVTSKCGSLGNIHHKPGGGQVEVKSEKLDKDRVQSKIIGSL</b> <b>DNITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTSR</b> <b>HLSNVSSTGSIDMVDSPLATLADEVSSASLAKQGL</b></p>
<b><u>Native sequence</u></b>	<p>Amino acids M1 – L441 (end) of human TAU. Residue M232 of the fusion protein is equivalent to M1 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 and <i>Not</i> I sites of pGEX6P-1

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### Nucleotide Sequence of insert

ggatccATGGCTGAGCCCCGCCAGGAGTTCGAAGTGATGGAAGATCACGC  
TGGGACGTACGGGTTGGGGGACAGGAAAGATCAGGGGGGCTACACCATGC  
ACCAAGACCAAGAGGGTGACACGGACGCTGGCCTGAAAGAATCTCCCCTG  
CAGACCCCCACTGAGGACGGATCTGAGGAACCGGGCTCTGAAACCTCTGA  
TGCTAAGAGCACTCCAACAGCGGAAGATGTGACAGCACCCCTTAGTGGATG  
AGGGAGCTCCCGGCAAGCAGGCTGCCGCGCAGCCCCACACGGAGATCCCA  
GAAGGAACCACAGCTGAAGAAGCAGGCATTGGAGACACCCCCAGCCTGGA  
AGACGAAGCTGCTGGTCACGTGACCCAAGCTCGCATGGTCAGTAAAAGCA  
AAGACGGGACTGGAAGCGATGACAAAAAAGCCAAGGGGGCTGATGGTAAA  
ACGAAGATCGCCACACCGCGGGGAGCAGCCCCCTCAGGCCAGAAGGGCCA  
GGCCAACGCCACCAGGATTCCAGCAAAAACCCGCCCCGCTCCAAAGACAC  
CACCCAGCTCTGGTGAACCTCCAAAATCAGGGGATCGCAGCGGCTACAGC  
AGCCCCGGCTCCCCAGGCACTCCCGGCAGCCGCTCCCGCACCCCCGTCCCT  
TCCAACCCACCCACCCGGGAGCCCAAGAAGGTGGCAGTGGTCCGTACTC  
CACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGCCCCCGTG  
CCCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAA  
CCTGAAGCACCAGCCGGGAGGCGGGAAGGTGCAGATAATTAATAAGAAGC  
TGGATCTTAGCAACGTCCAGTCCAAGTGTGGCTCAAAGGATAATATCAA  
CACGTCCCGGGAGGCGGCAGTGTGCAAATAGTCTACAAACCAGTTGACCT  
GAGCAAGGTGACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAAC  
CAGGAGGTGGCCAGGTGGAAGTAAAATCTGAGAAGCTTGACTTCAAGGAC  
AGAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCACCCACGTCCCTGG  
CGGAGGAAATAAAAAGATTGAAACCCACAAGCTGACCTTCCGCGAGAACG  
CCAAAGCCAAGACAGACCACGGGGCGGAGATCGTGTACAAGTCGCCAGTG  
GTGTCTGGGGACACGTCTCCACGGCATCTCAGCAATGTCTCCTCCACCGG  
CAGCATCGACATGGTAGACTCGCCCCAGCTCGCCACGCTAGCTGACGAGG  
TGTCTGCCTCCCTGGCCAAGCAGGGTTTGtgagcggccgc

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