

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of TIFA [2 - 184]

<u>Enzyme description:-</u>	TIFA [2 – 184]
<u>Clone number:-</u>	DU 4241
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 48, 106.86 daltons
Average Mass 48, 138.12 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.38

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

TIFA [2 – 184]

<u>Protein</u>	TIFA [1 – 184]
<u>Clone number</u>	DU 4241
<u>Species</u>	Human
<u>Accession number</u>	NM_052864.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSTSFEDADTEETVTC LQMTVYHPGQLQCGIFQSISFNREKLPSSEVVKFGRNSNICHYTFQDKQ VSRVQFSLQLFKKFNSSVLSFEIKNMSKKTNLIVDSRELGYLNKMDLPY RCMVRFGFYQFLMEKEDGESLEFFETQFILSPRSLLOENNWPPIRPIE YGTYSLCSSQSSSPTMDENES</p>
<u>Native sequence</u>	<p>Amino acids T2 – S184 (end) of human TIFA. Residue T232 of the fusion protein is equivalent to T2 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pGEX6P-1

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Complete
Nucleotide
Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATAACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCT
GGGATCCACCAGTTTTGAAGATGCTGACACAGAAGAGACAGTAACTTGT
CTCCAGATGACGGTTTACCATCCTGGCCAGTTGCAGTGTGGAATATTTT
AGTCAATAAGTTTTAACAGAGAGAACTCCCTTCCAGCGAAGTGGTGAA
ATTTGGCCGAAATCCAACATCTGTCATTATACTTTTCAGGACAAACAG
GTTTCCCGAGTTCAGTTTTCTCTGCAGCTGTTTAAAAAATTCAACAGCT
CAGTTCTCTCCTTTGAAATAAAAAATATGAGTAAAAAGACCAATCTGAT
CGTGGACAGCAGAGAGCTGGGCTACCTAAATAAAATGGACCTGCCATAC
AGGTGCATGGTCAGATTCGGAGAGTATCAGTTTCTGATGGAGAAGGAAG
ATGGCGAGTCATTGGAATTTTTTGGAGACTCAATTTATTTTATCTCCAAG
ATCACTCTTGCAAGAAAACAACTGGCCACCACACAGGCCCATACCGGAG
TATGGCACTTACTCGCTCTGCTCCTCCCAAAGCAGTTCTCCGACAGAAA
TGGATGAAAATGAGTCAtgagcggccgc