

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of TIFA T9A [2 - 184]

Enzyme description:- TIFA T9A [2 – 184]

Clone number:- DU 65850

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 48, 076.85 daltons

Average Mass 48, 108.09 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 T9A [2 – 184]

<u>Protein</u>	TIFA T9A [2 – 184]
<u>Clone number</u>	DU 65850
<u>Species</u>	Human
<u>Accession number</u>	NM_052864.2
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSTSFEDADAEETVTC LQMTVYHPGQLQCGIFQSISFNREKLPSSEVVKFGRNSNICHYTFQDKQ VSRVQFSLQLFKKFNSSVLSFEIKNMSKKTNLIVDSRELGYLNKMDLPY RCMVRFG EYQFLMEKEDGESLEFFETQF ILSPRSLLOENNWPPIRPIPE YGTYS LCSSQSSSPTEMDENES</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> <p>The enzyme has an T9A mutation. Residue T9 is equivalent to A239 of the fusion protein.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVL FQGP</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
CTCGACTTCTTTTGGAAATATCTTGAAGAAAAATATGAAGAGCATTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATAACGTTATATAGCTGACAAGCACAAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCTGGATGCGTTCCTCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCT
GGGATCCACCAGTTTTGAAGATGCTGACgCAGAAGAGACAGTAACTTGT
CTCCAGATGACGGTTTACCATCCTGGCCAGTTGCAGTGTGGAATATTTT
AGTCAATAAGTTTTAACAGAGAGAACTCCCTTCCAGCGAAGTGGTGAA
ATTTGGCCGAAATTCCAACATCTGTCATTATACTTTTCAGGACAAACAG
GTTTCCCGAGTTCAGTTTTCTCTGCAGCTGTTTAAAAAATTCAACAGCT
CAGTTCTCTCCTTTGAAATAAAAAATATGAGTAAAAAGACCAATCTGAT
CGTGGACAGCAGAGAGCTGGGCTACCTAAATAAAATGGACCTGCCATAC
AGGTGCATGGTCAGATTCGGAGAGTATCAGTTTCTGATGGAGAAGGAAG
ATGGCGAGTCATTGGAATTTTTTTGAGACTCAATTTATTTTATCTCCAAG
ATCACTCTTGCAAGAAAACAACCTGGCCACCACACAGGCCCATACGGAG
TATGGCACTTACTCGCTCTGCTCCTCCCAAAGCAGTTCTCCGACAGAAA
TGGATGAAAATGAGTCA_{tga}