Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of TIFA T9A T177A [2 - 184]

Enzyme description:- TIFA T9A T177A [2 – 184]

Clone number:- DU 71066

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 48, 046.84 daltons
Average Mass 48, 078.07 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 T177A [2 – 184]

Protein	TIFA T9A T177A [2 – 184]
Clone number	DU 71066
Species	Human
Accession number	NM_052864.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**TSFEDADAEETVTC** **LQMTVYHPGQLQCGIFQSISFNREKLPSSEVVKFGRNSNICHYTFQDKQ** **VSRVQFSLQLFKKFNSSVLSFEIKNMSKKTNLIVDSRELGYLNKMDLPY** **RCMVRFGEYQFLMEKEDGESLEFFETQFILSPRSLLQENNWPPHRPIPE** **YGTYSLCSSQSSSPAEMDENES**
Native sequence	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. The enzyme has an T9A and T177A mutation. Residue T9 is equivalent to A239 of the fusion protein and Residue T177 is equivalent to A407 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1 sites of pGEX6P-1

Complete Nucleotide Sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTT
TGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAAT
GGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGG
TGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATG
TTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGAT
ATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTT
TCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACATATT
TAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTA
TACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTG
AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCA
GGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGAAGTTCT
GTTCCAGGGGCCCCTGGGATCCACCAGTTTTGAAGATGCTGACGCAGAAGAGACAGTAAC
TTGTCTCCAGATGACGGTTTACCATCCTGGCCAGTTGCAGTGTGGAATATTTCAGTCAATA
AGTTTTAACAGAGAGAAACTCCCTTCCAGCGAAGTGGTGAAATTTGGCCGAAATTCCAACA
TCTGTCATTATACTTTTCAGGACAAACAGGTTTCCCGAGTTCAGTTTTCTCTGCAGCTGTTT
AAAAAATTCAACAGCTCAGTTCTCTCCTTTGAAATAAAAAATATGAGTAAAAAGACCAATCT
GATCGTGGACAGCAGAGAGCTGGGCTACCTAAATAAAATGGACCTGCCATACAGGTGCAT
GGTCAGATTCGGAGAGTATCAGTTTCTGATGGAGAAGGAAGATGGCGAGTCATTGGAATT
TTTTGAGACTCAATTTATTTTATCTCCAAGATCACTCTTGCAAGAAAACAACTGGCCACCAC
ACAGGCCCATACCGGAGTATGGCACTTACTCGCTCTGCTCCTCCCAAAGCAGTTCTCCGG
CAGAAATGGATGAAAATGAGTCAtga