Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of TIFA T9A T165A T177A [2 - 184]

Enzyme description:- TIFA T9A T165A T177A [2 – 184]

Clone number:- DU 71092

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 48, 016.83 daltons
Average Mass 48, 048.04 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 T165A T177A [2 – 184]

Protein	TIFA T9A T165A T177A [2 – 184]
Clone number	DU 71092
Species	Human
Accession number	NM_052864.2
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**TSFEDADAEETVTC** **LQMTVYHPGQLQCGIFQSISFNREKLPSSEVVKFGRNSNICHYTFQDKQ** **VSRVQFSLQLFKKFNSSVLSFEIKNMSKKTNLIVDSRELGYLNKMDLPY** **RCMVRFGEYQFLMEKEDGESLEFFETQFILSPRSLLQENNWPPHRPIPE** **YGAYSLCSSQSSSPAEMDENES**
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, T165A and T177A mutation. Residue T9 is equivalent to A239 of the fusion protein, residue T165 is equivalent to A395 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

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCA
CTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTA
TGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGT
TTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAA
CACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTT
GGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCG
GTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAA
AATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTAT
ACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAA
AAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGC
AAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTG
GCGACCATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCT
GGGATCCACCAGTTTTGAAGATGCTGACGCAGAAGAGACAGTAACTTGT
CTCCAGATGACGGTTTACCATCCTGGCCAGTTGCAGTGTGGAATATTTC
AGTCAATAAGTTTTAACAGAGAGAAACTCCCTTCCAGCGAAGTGGTGAA
ATTTGGCCGAAATTCCAACATCTGTCATTATACTTTTCAGGACAAACAG
GTTTCCCGAGTTCAGTTTTCTCTGCAGCTGTTTAAAAAATTCAACAGCT
CAGTTCTCTCCTTTGAAATAAAAAATATGAGTAAAAAGACCAATCTGAT
CGTGGACAGCAGAGAGCTGGGCTACCTAAATAAAATGGACCTGCCATAC
AGGTGCATGGTCAGATTCGGAGAGTATCAGTTTCTGATGGAGAAGGAAG
ATGGCGAGTCATTGGAATTTTTTGAGACTCAATTTATTTTATCTCCAAG
ATCACTCTTGCAAGAAAACAACTGGCCACCACACAGGCCCATACCGGAG
TATGGCGCTTACTCGCTCTGCTCCTCCCAAAGCAGTTCTCCGGCAGAAA
TGGATGAAAATGAGTCAtga