Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active TGFBR1 T204D [200 – 501]

Enzyme description:- TGFBR1 T204D [200 – 501]

Clone number:- DU 33547

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- Glutathione Sepharose

Calculated molecular mass:-
Monoisotopic 61, 702.43 daltons
Average Mass 61, 742.36 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.97

Purity:- 80 %

Activation protocol:- Constitutively active

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- –70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
RRKVLTQMGSPSIRCSS*VS [where S* is phosphor Serine]
Final concentration: 300 μM

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Clone Data Sheet

TGFBR1 T204D [200 – 501]

Protein	TGFBR1 T204D [200 - 501]
Clone number	DU 38666
Species	Human
Accession number	NM_004612
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGSM**TIARDIVLQESIGKGRFGEVWRGKWRGEEVAVKIFSSR** **EERSWFREAEIYQTVMLRHENILGFIAADNKDNGTWTQLWLVSDY** **HEHGSLFDYLNRYTVTVEGMIKLALSTASGLAHLHMEIVGTQGKP** **AIAHRDLKSKNILVKKNGTCCIADLGLAVRHDSATDTIDIAPNHR** **VGTKRYMAPEVLDDSINMKHFESFKRADIYAMGLVFWEIARRCSI** **GGIHEDYQLPYYDLVPSDPSVEEMRKVVCEQKLRPNIPNRWQSCE** **ALRVMAKIMRECWYANGAARLTALRIKKTLSQLSQQEGIKM**
Native sequence	Amino acids T200 – M501 (end) of human TGFBR1. Residue T233 of the fusion protein is equivalent to T200 of the native enzyme. The GST tag is located at residues 1 - 220. The enzyme has a T204D mutation in order to mimic phosphorylation of the enzyme. Residues T204 is equivalent to D237 of the fusion protein.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bgl*1 and *Not*I to *Bam*H1 and *Not*1 sites of pFB-GST

Nucleotide sequence of insert

agatctatgACAATTGCGAGAGATATTGTGTTACAAGAAAGCATT
GGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGGGGA
GAAGAAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTCG
TGGTTCCGTGAGGCAGAGATTTATCAAACTGTAATGTTACGTCAT
GAAAACATCCTGGGATTTATAGCAGCAGACAATAAAGACAATGGT
ACTTGGACTCAGCTCTGGTTGGTGTCAGATTATCATGAGCATGGA
TCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGA
ATGATAAAACTTGCTCTGTCCACGGCGAGCGGTCTTGCCCATCTT
CACATGGAGATTGTTGGTACCCAAGGAAAGCCAGCCATTGCTCAT
AGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAACT
TGCTGTATTGCAGACTTAGGACTGGCAGTAAGACATGATTCAGCC
ACAGATACCATTGATATTGCTCCAAACCACAGAGTGGGAACAAAA
AGGTACATGGCCCCTGAAGTTCTCGATGATTCCATAAATATGAAA
CATTTTGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTA
GTATTCTGGGAAATTGCTCGACGATGTTCCATTGGTGGTATTCAT
GAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCTGACCCA
TCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAGG
CCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTA
ATGGCTAAAATTATGAGAGAATGTTGGTATGCCAATGGAGCAGCT
AGGCTTACAGCATTGCGGATTAAGAAAACATTATCGCAACTCAGT
CAACAGGAAGGCATCAAAATGtaagcggccgc