

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TGFBR1 T204D [200 – 501]

<u>Enzyme description:-</u>	TGFBR1 T204D [200 – 501]
<u>Clone number:-</u>	DU 33547
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	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:-

RRKVL^TQMGSPSIRCSS^{S*}VS [where S* is phosphor Serine]
Final concentration: 300 μM

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Clone Data Sheet

TGFBR1 T204D [200 – 501]

<u>Protein</u>	TGFBR1 T204D [200 - 501]
<u>Clone number</u>	DU 38666
<u>Species</u>	Human
<u>Accession number</u>	NM_004612
<u>Tags</u>	N-terminal GST
<u>Baculovirus expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSMTIARDIVLQESIGKGRFGEVWRGKWRGEEVAVKIFSSR EERSWFREAEIYQTVMLRHENILGFIAADNKDNGTWTQLWLVS DIYHEGSLFDYLNRYTVTVEGMIKLALSTASGLAHLHMEIVGTQGKP AIAHRDLKSKNILVKKNGTCCIADLGLAVRHDSATDTIDIAPNHR VGTKRYMAPEVLDD SINMKHFESFKRADIYAMGLVFWEIARRCSI GGIHEDYQLPYYDLVPSDPSVEEMRKVVCEQKLRPNIPNRWQSC ALRVMKIMRECWYANGAARLTALRIKKTLSQLSQQEGIKM</p>
<u>Native sequence</u>	<p>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. Residue T204 is equivalent to D237 of the fusion protein.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bgl</i> I and <i>Not</i> I to <i>Bam</i> H1 and <i>Not</i> I sites of pFB-GST

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Nucleotide sequence of insert

agatctatgACAATTGCGAGAGATATTGTGTTACAAGAAAGCATT
GGCAAAGGTCGATTTGGAGAAGTTTGGAGAGGAAAGTGGCGGGGA
GAAGAAGTTGCTGTTAAGATATTCTCCTCTAGAGAAGAACGTTCCG
TGGTTCCGTGAGGCAGAGATTTATCAAACCTGTAATGTTACGTCAT
GAAAACATCCTGGGATTTATAGCAGCAGACAATAAAGACAATGGT
ACTTGGACTCAGCTCTGGTTGGTGTGAGATTATCATGAGCATGGA
TCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGGA
ATGATAAAACTTGCTCTGTCCACGGCGAGCGGTCTTGCCCATCTT
CACATGGAGATTGTTGGTACCCAAGGAAAGCCAGCCATTGCTCAT
AGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAAC
TGCTGTATTGCAGACTTAGGACTGGCAGTAAGACATGATTCAGCC
ACAGATAACCATTGATATTGCTCCAAACCACAGAGTGGGAACAAAA
AGGTACATGGCCCCTGAAGTTCTCGATGATTCCATAAATATGAAA
CATTTTGAATCCTTCAAACGTGCTGACATCTATGCAATGGGCTTA
GTATTCTGGGAAATTGCTCGACGATGTTCCATTGGTGGTATTCAT
GAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCTGACCCA
TCAGTTGAAGAAATGAGAAAAGTTGTTTGTGAACAGAAGTTAAGG
CCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAGAGTA
ATGGCTAAAATTATGAGAGAATGTTGGTATGCCAATGGAGCAGCT
AGGCTTACAGCATTGCGGATTAAGAAAACATTATCGCAACTCAGT
CAACAGGAAGGCATCAAAATGtaagcggccgc