

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of TANK [1 – 425]

Enzyme description:- TANK [1 – 425]

Clone number:- DU 12685

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 74, 620.56 daltons

Average Mass 74, 667.93 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.56

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

TANK [1 – 425]

<u>Protein</u>	TANK [1 – 425]
<u>Clone number</u>	DU 12685
<u>Species</u>	Human
<u>Accession number</u>	BC067779.1
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSMDKNIGEQLNKAYE AFRQACMDRDSAVKELQOKTENYEQRIREQQEQLSLQOTIIDKLKSOLL LVNSTQDNNYGCVPILLEDSETRKNNLTLDQPQDKVISGIAREKLPKVR QEVSSPRKETSARSLGSPLLHERGNIKTFWDLKEEFHKICMLAKAQKD HLSKLNIPDTATETQCSVPIQCTDKTDKQEQALFKPOAKDDINRGAPSI SVTPRGLCRDEEDTSFESLSKFNKFPMDNDSTFLHSTPERPGILSPA TSEVVCQEQKFNMEFRDNPNGNFVKTEETLFEIQGIDPIASAIQNLKTTDK TKPSNLVNTCIRTLDRAACLPPGDHNALYVNSFPLLDPSDAPFPSLDS PGKAIRGPQQPIWKFPNQSDSVVLSGTDSELHIPRVCEFCQAVFPPS ITSRGDFLRHLNSHFNGET</p>
<u>Native sequence</u>	<p>Amino acids M1 – T425 (end) of human TANK. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGP</u>) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Sal</i> I sites into pGEX6P-1

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Nucleotide Sequence of Insert:

ggatccATGGATAAAAACATTGGCGAGCAACTCAATAAAGCGTATGAAGCCTTCCGGCAGGCATGC
ATGGATAGAGATTCTGCAGTAAAAGAATTACAGCAAAGACTGAGAACTATGAGCAGAGAATACGT
GAACAACAGGAACAGCTGTCAC TTCAACAGACTATTATTGACAAGCTAAAATCTCAGTTACTTCTT
GTGAATTCCACTCAAGATAACAATTATGGCTGTGTTCCCTCTGCTTGAAGACAGTGAAACAAGAAAG
AATAATTTGACTCTTGATCAGCCACAAGATAAAGTGATTTTCAGGAATAGCAAGAGAAAACTACCA
AAGGTAAGAAGACAAGAGGTTTCTTCTCCTAGAAAAGAAACTTCAGCAAGGAGTCTTGGCAGTCCT
TTGCTCCATGAAAGGGGTAATATAGAGAAGACTTTTCTGGGATCTGAAAGAAGAATTTCATAAAATA
TGCATGCTAGCAAAAGCACAGAAAGACCACTTAAGCAAACCTAATATACCAGACACTGCAACTGAA
ACACAGTGCTCTGTGCC TATACAGTGTACGGATAAAACAGATAAACAAGAAGCGCTGTTTAAGCCT
CAGGCTAAAGATGATATAAATAGAGGTGCACCATCCATCACATCTGTACACCAAGAGGACTGTGC
AGAGATGAGGAAGACACCTCTTTTGAATCACTTTCTAAATTC AATGTCAAGTTTCCACCTATGGAC
AATGACTCAACTTTCTTACATAGCACTCCAGAGAGACCCGGCATCCTTAGTCCTGCCACGCTGAG
GTAGTGTGCCAAGAGAAATTTAATATGGAGTTCAGAGACAACCCAGGGA ACTTTGTTAAAACAGAA
GAACTTTATTTGAAATTCAGGGAATTGACCCCATAGCTTCAGCTATACAAAACCTTAAAACAACT
GACAAAACAAAGCCCTCAAATCTCGTAAACACTTGTATCAGGACA ACTCTGGATAGAGCTGCGTGT
TTGCCACCTGGAGACCATAATGCATTATATGTAAATAGCTTCCCACTTCTGGACCCATCTGATGCA
CCTTTTCCCTCACTCGATTCCCCGGGAAAAGCAATCCGAGGACCACAGCAGCCCATTTGGAAGCCC
TTTCCTAATCAAGACAGTGACTCGGTGGTACTAAGTGGCACAGACTCAGAACTGCATATACCTCGA
GTATGTGAATTTCTGTCAAGCAGTTTTCCCACTCCATTACATCCAGGGGGGATTTCCCTTCGGCAT
CTTAATTCACACTTCAATGGAGAGACTtaagtcgac

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