

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of TAB3 [2 – 712]

<u>Enzyme description:-</u>	TAB3 [2 - 712]
<u>Clone number:-</u>	DU3033
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH Sepharose

Calculated molecular mass:-

Monoisotopic 105, 279.17 daltons
Average Mass 105, 345.83 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 8.15

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

TAB3 [2 - 712]

<u>Protein</u>	TAB3 [2 - 712]
<u>Clone number</u>	DU 3033
<u>Species</u>	Human
<u>Accession number</u>	NM_152787.3
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLVLFQGPLGSAQSSPQLDIQVLHD LRQRFPEIPEGVVSQCMLQNNNLEACCRALSQESSKYLMEYHSPDDN RMNRNRLHINLGIHSPSSYHPGDGAQLNGGRTL VHSSSDGHIDPOHAA GKQLICLVQEPHSAPAVVAATPNYNPFFMNEQNRSAATPPSQPPQPS MQTGMNPSAMQGPSPPPPPSYMHIPRYSTNPI TVTVSQNLPSGQTVPR ALQILPQIPSNLYGSPGSIYIRQTSQSSSGRQTPQSTPWQSSPQGPVPH YSQRPLPVYPHQONYQPSQYSPKQQOIQSAYHSPPPSQCPSPFSSPQH QVQPSQLGHIFMPPSPSTTPPHYQOGPPSYQKQGSHSVAYLPTASSL SKGSMKKIEITVEPSQRPGTAINRSPSPISNQPSPRNQHSLYTATPPS SSPSRGISSQPKPPFSVNPVYITYTQPTGSPCTPSPSPRVIPNPTTVFK ITVGRATTENLLNLVDQEERSAAPEIQPISVIPGSGGEKGS HKYQRSS SSGSDDYAYTQALLHQ RARMERLAKQLKLEKEELERLKSEVNGMEHDL MQRRLRRVSC TTAIPTPEEMTRLRSMNRQLQINVDCTLKEVDLLQSRGN FDPKAMNNFYDNIEGPPVVPKPSKKDSSDPCTIERKARRISVTSKVQA DIHDTQAAA ADEHRTGSTQSPRTQPRDEDEYEGAPWNCDSCTFLNHPALN RCEQCEMPRYT</p>
<u>Native sequence</u>	<p>Amino acids A2 – T712 (end) of human TAB3. Residue A232 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220.</p>
<u>Protease cleavage</u>	PreScission (LEVLVFGGP) residues 221 - 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pGEX6P-1

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Nucleotide Sequence of insert

ggatccGCGCAAAGCAGCCCACAGCTTGATATTCAGGTTCTCCATGATCTTCGACAACGTTTCCCT
GAAATTCAGAGGGCGTGGTGTCTCAGTGCATGTTACAGAATAACAACAATCTTGAAGCCTGTTGC
CGAGCCCTTTCCAGGAGAGTAGCAAATACTTATATATGGAATACCATAGTCCAGATGACAATAGG
ATGAATAGAAATCGCCTTTTACATATTAACCTGGGTATCCATTCTCCTAGTAGCTATCACCCAGGA
GATGGAGCCCAACTTAATGGTGGTTCGAACACTGGTACATAGCTCAAGTGATGGACATATTGATCCT
CAGCATGCAGCAGGTAAACAGCTGATATGTTTAGTTCAAGAACCACACTCAGCTCCAGCTGTTGTT
GCTGCTACTCCCAACTACAATCCATTTTATGAACGAACAGAACAGAAGTGCAGCTACTCCTCCT
TCACAACCACCTCAACAGCCATCTTCCATGCAAACAGGAATGAATCCGTCTGCTATGCAAGGGCCT
TCACCACCACCGCCACCTCCTTCATACATGCACATACTCGGTATAGTACAAATCCAATTACTGTT
ACAGTATCCCAGAACCTCCCTTCTGGACAGACTGTACCAAGAGCTTTACAAATTCTTCCACAAATT
CCAAGCAATCTCTATGGGTCTCCTGGTCTATTTATATTAGACAGACATCTCAGAGTTCATCAGGA
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CAGATCCCTCAGTCTGCTTACCATTACCCACCTCCTTCTCAATGTCTTACCCTTCAGCTCTCCA
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GAGCAGTGCAGATGCCACGGTACACCTgaattc

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