

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Bruton agammaglobulinemia Tyrosine Kinase [2 - 659]

<u>Enzyme description:-</u>	BTK [2 - 659]
<u>Clone number:-</u>	DU 12110
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	3 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	79, 470.73 daltons
Average Mass	79, 521.68 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.06
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.	
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
KVEKIGEGTYGVVYK	Final concentration: 300 µM
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

Bruton agammaglobulinemia Tyrosine Kinase [2 - 659]

<u>Protein</u>	BTK [2 - 659]
<u>Clone number</u>	DU 12110
<u>Species</u>	Human
<u>Accession number</u>	NP_00052.1
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHDYDIPTTENLYFQGAMGSAAVILESIFLKRSQQKKKTSP LNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDVEKITCVETVVPEK NPPPERQIPRRGEESSEMEQISIIERFPYPFQVVYDEGPLYVFSPTTEL RKRWIHQ LKNVIRYNSDLVQKYHPCFWIDGOYLCCSQ TAKNAMGCQILE NRNGSLKPGSSHRKTKKPLPPTPEEDQILKKPLPPEPAAAPVSTSELKK VVALYDYMPMNANDLQLRKGDEYFILEESNLPWWRARDKNGQEGYIPSN YVTEAEDSIEMYEWYSKHMTRSQA EQLLKQEGKEGGFIVRDSSKAGKYT VSVFAKSTGDPQGVIRHYVVCSTPQSQYYLAEKHLFSTIPELINYHQHN SAGLISRLKYPVSQQNKNAPSTAGLGYGSWEIDPKDLTFLKELGTGQFG VVKYGKWRGQYDVAIKMIKEGSMSEDEFIEEAKVMMNLSHEKLVQLYGV CTKQRPIFIITEYMANGCLLNLYLREMRHRFQTQQLLEMCKDVCEAMEYL ESKQFLHRDLAARNCLVNDQGVVKVSDFGLSRYVLDDEYTSSVGSKFPV RWSPEVLMYSKFSSKSDIWA FGVL MWEIYSLGKMPYERFTNSETAEHI AQGLRLYRPHLASEKVYTIMYSCWHEKADERPTFKILLSNILDVMDEES
<u>Native sequence</u>	Amino acids A2 – S659 (end) of human BTK. Residue A29 of the fusion protein is equivalent to A2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 - 24
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> I sites of pFastBAC HTb

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Nucleotide

Sequence of insert

ggatccGCCGCAGTGATTCTGGAGAGCATCTTTCTGAAGCGATCCCAAC
AGAAAAAGAAAACATCACCTCTAAACTTCAAGAAGCGCCTGTTTCTCTT
GACCGTGCACAACTCTCCTACTATGAGTATGACTTTGAACGTGGGAGA
AGAGGCAGTAAGAAGGGTTCAATAGATGTTGAGAAGATCACTTGTGTTG
AAACAGTGGTTCCCTGAAAAAATCCTCCTCCAGAAAGACAGATTCCGAG
AAGAGGTGAAGAGTCCAGTGAAATGGAGCAAATTTCAATCATTGAAAGG
TTCCCTTATCCCTTCCAGGTTGTATATGATGAAGGGCCTCTCTACGTCT
TCTCCCCAACTGAAGAACTAAGGAAGCGGTGGATTACACAGCTCAAAAA
CGTAATCCGGTACAACAGTGATCTGGTTCAGAAATATCACCTTGCTTC
TGGATCGATGGGCAGTATCTCTGCTGCTCTCAGACAGCCAAAAATGCTA
TGGGCTGCCAAATTTTGGAGAACAGGAATGGAAGCTTAAAACCTGGGAG
TTCTCACCGGAAGACAAAAAAGCCTCTTCCCCAACGCCTGAGGAGGAC
CAGATCTTGAAAAAGCCACTACCGCCTGAGCCAGCAGCAGCACCAGTCT
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GAATGCAAATGATCTACAGCTGCGGAAGGGTGATGAATATTTTATCTTG
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AATGTATGAGTGGTATTCCAAACACATGACTCGGAGTCAGGCTGAGCAA
CTGCTAAAGCAAGAGGGGAAAGAAGGAGGTTTCATTGTCAGAGACTCCA
GCAAAGCTGGCAAATATACAGTGTCTGTGTTTGCTAAATCCACAGGGGA
CCCTCAAGGGGTGATACGTCATTATGTTGTGTGTTCCACACCTCAGAGC
CAGTATTACCTGGCTGAGAAGCACCTTTTCAGCACCATCCCTGAGCTCA
TTAACTACCATCAGCACAACTCTGCAGGACTCATATCCAGGCTCAAATA
TCCAGTGTCTCAACAAAAACAAGAATGCACCTTCCACTGCAGGCCCTGGGA
TACGGATCATGGGAAATTGATCCAAAGGACCTGACCTTCTTGAAGGAGC
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GAATTCATTGAAGAAGCCAAAGTCATGATGAATCTTTCCCATGAGAAGC
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CACTGAGTACATGGCCAATGGCTGCCTCCTGAACTACCTGAGGGAGATG
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GTGAAGCCATGGAATACCTGGAGTCAAAGCAGTTCCTTCACCGAGACCT
GGCAGCTCGAACTGTTTGGTAAACGATCAAGGAGTTGTTAAAGTATCT
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TAGGCTCCAAATTTCCAGTCCGGTGGTCCCCACCGGAAGTCCTGATGTA
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AAAGCAGATGAGCGTCCCACTTTCAAATTCCTTCTGAGCAATATTCTAG
ATGTCATGGATGAAGAATCctgagcgccgc