University of Dundee

Standard Operating Procedure

Preparation of Bruton agammaglobulinemia Tyrosine Kinase [2 - 659]

Enzyme description:- BTK [2 - 659]

Clone number:- DU 12110

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni2+-NTA agarose

Expression level:- 3 mg/L

Calculated molecular mass:-

Monoisotopic 79, 470.73 daltons
Average Mass 79, 521.68 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.06

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C [Long term stability to be determined]

Assay:- Standard filter binding assay

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

Substrate:-
KVEKIGEGTYGVVYK Final concentration: 300 μM

Specific activity range:- To be determined

Clone Data Sheet - Bruton agammaglobulinemia Tyrosine Kinase [2 - 659]

Protein	BTK [2 - 659]
Clone number	DU 12110
Species	Human
Accession number	NP_00052.1
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**AAVILESIFLKRSQQ KKKTSPLNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDV EKITCVETVVPEKNPPPERQIPRRGEESSEMEQISIIERFPYPFQ VVYDEGPLYVFSPTEELRKRWIHQLKNVIRYNSDLVQKYHPC FWIDGQYLCCSQTAKNAMGCQILENRNGSLKPGSSHRKTKK PLPPTPEEDQILKKPLPPEPAAAPVSTSELKKVVALYDYMPM NANDLQLRKGDEYFILEESNLPWWRARDKNGQEGYIPSNYV TEAEDSIEMYEWYSKHMTRSQAEQLLKQEGKEGGFIVRDSS KAGKYTVSVFAKSTGDPQGVIRHYVVCSTPQSQYYLAEKHLF STIPELINYHQHNSAGLISRLKYPVSQQNKNAPSTAGLGYGSW EIDPKDLTFLKELGTGQFGVVKYGKWRGQYDVAIKMIKEGS MSEDEFIEEAKVMMNLSHEKLVQLYGVCTKQRPIFIITEYMA NGCLLNYLREMRHRFQTQQLLEMCKDVCEAMEYLESKQFL HRDLAARNCLVNDQGVVKVSDFGLSRYVLDDEYTSSVGSKFP VRWSPPEVLMYSKFSSKSDIWAFGVLMWEIYSLGKMPYERF TNSETAEHIAQGLRLYRPHLASEKVYTIMYSCWHEKADERPT FKILLSNILDVMDEES**
Native sequence	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.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	BamH1 and Not1 sites of pFastBAC HTb

Nucleotide sequence of insert

ggatccGCCGCAGTGATTCTGGAGAGCATCTTTCTGAAGCGATCC
CAACAGAAAAAGAAAACATCACCTCTAAACTTCAAGAAGCGC
CTGTTTCTCTTGACCGTGCACAAACTCTCCTACTATGAGTATG
ACTTTGAACGTGGGAGAAGAGGCAGTAAGAAGGGTTCAATAG
ATGTTGAGAAGATCACTTGTGTTGAAACAGTGGTTCCTGAAA
AAAATCCTCCTCCAGAAAGACAGATTCCGAGAAGAGGTGAAG
AGTCCAGTGAAATGGAGCAAATTTCAATCATTGAAAGGTTCC
CTTATCCCTTCCAGGTTGTATATGATGAAGGGCCTCTCTACGT
CTTCTCCCCAACTGAAGAACTAAGGAAGCGGTGGATTCACCA
GCTCAAAAACGTAATCCGGTACAACAGTGATCTGGTTCAGAA
ATATCACCCTTGCTTCTGGATCGATGGGCAGTATCTCTGCTGC
TCTCAGACAGCCAAAAATGCTATGGGCTGCCAAATTTTGGAG
AACAGGAATGGAAGCTTAAAACCTGGGAGTTCTCACCGGAAG
ACAAAAAAGCCTCTTCCCCCAACGCCTGAGGAGGACCAGATC
TTGAAAAAGCCACTACCGCCTGAGCCAGCAGCAGCACCAGTC
TCCACAAGTGAGCTGAAAAAGGTTGTGGCCCTTTATGATTACA
TGCCAATGAATGCAAATGATCTACAGCTGCGGAAGGGTGATG
AATATTTTATCTTGGAGGAAAGCAACTTACCATGGTGGAGAG
CACGAGATAAAAATGGGCAGGAAGGCTACATTCCTAGTAACT
ATGTCACTGAAGCAGAAGACTCCATAGAAATGTATGAGTGGT
ATTCCAAACACATGACTCGGAGTCAGGCTGAGCAACTGCTAA
AGCAAGAGGGGAAAGAAGGAGGTTTCATTGTCAGAGACTCCA
GCAAAGCTGGCAAATATACAGTGTCTGTGTTTGCTAAATCCAC
AGGGGACCCTCAAGGGGTGATACGTCATTATGTTGTGTGTTCC
ACACCTCAGAGCCAGTATTACCTGGCTGAGAAGCACCTTTTCA
GCACCATCCCTGAGCTCATTAACTACCATCAGCACAACTCTGC
AGGACTCATATCCAGGCTCAAATATCCAGTGTCTCAACAAAA
CAAGAATGCACCTTCCACTGCAGGCCTGGGATACGGATCATG
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GACTGGACAATTTGGGGTAGTGAAGTATGGGAAATGGAGAGG
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GTCTGAAGATGAATTCATTGAAGAAGCCAAAGTCATGATGAA
TCTTTCCCATGAGAAGCTGGTGCAGTTGTATGGCGTCTGCACC
AAGCAGCGCCCCATCTTCATCATCACTGAGTACATGGCCAATG
GCTGCCTCCTGAACTACCTGAGGGAGATGCGCCACCGCTTCCA
GACTCAGCAGCTGCTAGAGATGTGCAAGGATGTCTGTGAAGC
CATGGAATACCTGGAGTCAAAGCAGTTCCTTCACCGAGACCT
GGCAGCTCGAAACTGTTTGGTAAACGATCAAGGAGTTGTTAA
AGTATCTGATTTCGGCCTGTCCAGGTATGTCCTGGATGATGAA
TACACAAGCTCAGTAGGCTCCAAATTTCCAGTCCGGTGGTCCC
CACCGGAAGTCCTGATGTATAGCAAGTTCAGCAGCAAATCTG
ACATTTGGGCTTTTGGGGTTTTGATGTGGGAAATTTACTCCCT
GGGGAAGATGCCATATGAGAGATTTACTAACAGTGAGACTGC
TGAACACATTGCCCAAGGCCTACGTCTCTACAGGCCTCATCTG
GCTTCAGAGAAGGTATATACCATCATGTACAGTTGCTGGCATG

AGAAAGCAGATGAGCGTCCCACTTTCAAAATTCTTCTGAGCA
ATATTCTAG ATGTCATGGATGAAGAATCCtgagcggccgc