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Certificate of Analysis

BTK (E41K), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-773, 14-773-K, 14-773M
Parent Lot # D7MN055N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged, recombinant, full-length, human BTK containing the E41K mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Specific mutations within the pleckstrin homology (PH) domain of BTK results in human X-linked agammaglobulinemia (XLA). The E41K substitution, identified through random mutagenesis, is a PH domain gain-of-function mutation that has been shown to be associated with increased membrane localization and tyrosine phosphorylation of BTK. Using animal models, this mutation has been shown to arrest the development of immature B cells and to act as a tumour suppressor in B-cell linker protein (BLNK/SLP-65)-deficient hosts. (Li, *.et al*., Immunity,(1995);**2**:451-460 and Baraldi, E., *et al*., Structure,(1999);**7**:449-460).
Purity 62% by SDS-PAGE and Coomassie blue staining. MW = 78.4kDa.

Specific Activity (Parent lot# D7MN055N): 48U/mg, where one unit of BTK (E41K) activity is defined as 1nmol phosphate incorporated into 300µM LKBtide (LSNLYHQGKFLQTFCGSPLYRRR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.88mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

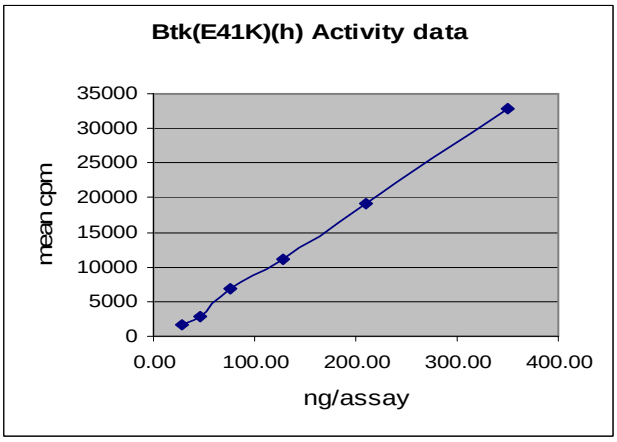
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

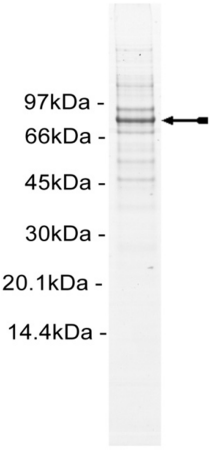
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 28–350ng of this lot of enzyme phosphorylated 300µM LKBtide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as BTK with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active BTK (E41K).

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **LKBtide (LSNLYHQGKFLQTFCGSPLYRRR):** Use at a final assay concentration of 300µM. Prepare a 3mM stock and add 2.5µl of stock per assay point.
3. **NaCl:** Use at a final assay concentration of 100mM. Make a 3M stock. Add 0.83µl of stock per assay point.
4. **Triton X-100:** Make up a 10% (w/v) stock. Add 2.5µl of stock per assay point.
5. **BTK (E41K), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 28–350ng per assay point.
6. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **LKBtide.**
3. Add **2.5µl (28–350ng) BTK (E41K), active**.
4. Add 0.83µl of 3M NaCl
5. Add 2.5µl of 10% (w/v) Triton X-100.
6. Add 1.67µl of dH_2O.
7. Add 10µl of diluted [γ-^{33}P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl aliquot onto the appropriate area of a P30 Filtermat.
11. Wash the filtermat three times for 5 minutes with 50mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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BTK (E41K) Sequence Information

Protein	human BTK (E41K)
Tags	*N*-terminal 6His
Native sequence	M16 of the recombinant protein is equivalent to M1 of human BTK
Accession number	GenBank NM_000061

Recombinant BTK amino acid sequence:

```
  1 MHHHHHHEFK GLRRRMAAVI LESIFLKRSQ QKKKTSPLNF KKRLFLLTVH KLSYYKYDFE
 61 RGRRGSKKGS IDVEKITCVE TVVPEKNPPP ERQIPRRGEE SSEMEQISII ERFPYPFQVV
121 YDEGPLYVFS PTEELRKRWI HQLKNVIRYN SDLVQKYHPC FWIDGQYLCC SQTAKNAMGC
181 QILENRNGSL KPGSSHRKTK KPLPPTPEED QILKKPLPPE PAAAPVSTSE LKKVVALYDY
241 MPMNANDLQL RKGDEYFILE ESNLPWWRAR DKNGQEGYIP SNYVTEAEDS IEMYEWYSKH
301 MTRSQAEQLL KQEGKEGGFI VRDSSKAGKY TVSVFAKSTG DPQGVIRHYV VCSTPQSQYY
361 LAEKHLFSTI PELINYHQHN SAGLISRLKY PVSQQNKNAP STAGLGYGSW EIDPKDLTFL
421 KELGTGQFGV VKYGKWRGQY DVAIKMIKEG SMSEDEFIEE AKVMMNLSHE KLVQLYGVCT
481 KQRPIFIITE YMANGCLLNY LREMRHRFQT QQLLEMCKDV CEAMEYLESK QFLHRDLAAR
541 NCLVNDQGVV KVSDFGLSRY VLDDEYTSSV GSKFPVRWSP PEVLMYSKFS SKSDIWAFGV
601 LMWEIYSLGK MPYERFTNSE TAEHIAQGLR LYRPHLASEK VYTIMYSCWH EKADERPTFK
661 ILLSNILDVM DEES
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Recombinant BTK nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcaaa ggcctacgtc gacgaatggc cgcagtgatt
  61 ctggagagca tctttctgaa gcgatcccaa cagaaaaaga aaacatcacc tctaaacttc
 121 aagaagcgcc tgtttctctt gaccgtgcac aaactctcct actataagta tgactttgaa
 181 cgtgggagaa gaggcagtaa gaagggttca atagatgttg agaagatcac ttgtgttgaa
 241 acagtggttc ctgaaaaaaa tcctcctcca gaaagacaga ttccgagaag aggtgaagag
 301 tccagtgaaa tggagcaaat ttcaatcatt gaaaggttcc cttatccctt ccaggttgta
 361 tatgatgaag ggcctctcta cgtcttctcc ccaactgaag aactaaggaa gcggtggatt
 421 caccagctca aaaacgtaat ccggtacaac agtgatctgg ttcagaaata tcacccttgc
 481 ttctggatcg atgggcagta tctctgctgc tctcagacag ccaaaaatgc tatgggctgc
 541 caaattttgg agaacaggaa tggaagctta aaacctggga gttctcaccg gaagacaaaa
 601 aagcctcttc ccccaacgcc tgaggaggac cagatcttga aaaagccact accgcctgag
 661 ccagcagcag caccagtctc cacaagtgag ctgaaaaagg ttgtggccct ttatgattac
 721 atgccaatga atgcaaatga tctacagctg cggaagggtg atgaatattt tatcttggag
 781 gaaagcaact taccatggtg gagagcacga gataaaaatg ggcaggaagg ctacattcct
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1021 gaccctcaag gggtgatacg tcattatgtt gtgtgttcca cacctcagag ccagtattac
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1441 aagcagcgcc ccatcttcat catcactgag tacatggcca atggctgcct cctgaactac
1501 ctgagggaga tgcgccaccg cttccagact cagcagctgc tagagatgtg caaggatgtc
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1561 tgtgaagcca tggaatacct ggagtcaaag cagttccttc accgagacct ggcagctcga
1621 aactgtttgg taaacgatca aggagttgtt aaagtatctg atttcggcct gtccaggtat
1681 gtcctggatg atgaatacac aagctcagta ggctccaaat ttccagtccg gtggtcccca
1741 ccggaagtcc tgatgtatag caagttcagc agcaaatctg acatttgggc ttttggggtt
1801 ttgatgtggg aaatttactc cctggggaag atgccatatg agagatttac taacagtgag
1861 actgctgaac acattgccca aggcctacgt ctctacaggc ctcatctggc ttcagagaag
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1981 attcttctga gcaatattct agatgtcatg gatgaagaat cctga
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Reviewed and approved by site quality representative.