

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 Ni²⁺/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.

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.

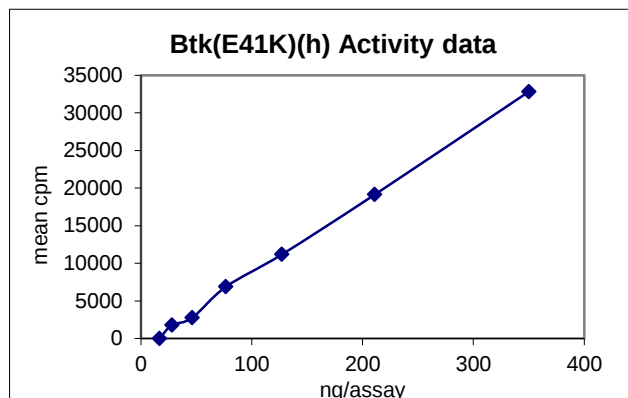
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

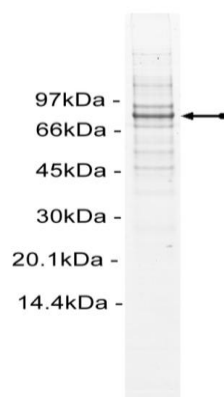
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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 BTK (E41K), active.

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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 300 μ M. Prepare a 3mM stock and add 2.5 μ l of stock per assay point.
3. **NaCl:** Use at a final assay concentration of 100mM. Make a 3M stock. Add 0.83 μ l of stock per assay point.
4. **Triton X-100:** Make up a 10% (w/v) stock. Add 2.5 μ l 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 -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **LKBtide**.
3. Add **2.5 μ l (28–350ng) BTk (E41K), active**.
4. Add 0.83 μ l of 3M NaCl
5. Add 2.5 μ l of 10% (w/v) Triton X-100.
6. Add 1.67 μ l of dH₂O.
7. Add 10 μ l of diluted [γ -³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
10. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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BTK (E41K) Sequence Information

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

Recombinant BTK amino acid sequence:

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1  MHHHHHHEFK  GLRRRMAAVI  LESIFLKRSQ  QKKKTSPLNF  KKRLFLLTVH  KLSYYKYDFE
61  RGRRGSKKGS  IDVEKITCVE  TVVPEKNPPP  ERQIPRRGEE  SSEMEQISII  ERFPPYFQVV
121 YDEGPLYVFS  PTEELRKRWI  HQLKNVIRYN  SDLVQKYHPC  FWIDGQYLCC  SQTAKNAMGC
181 QILENRNGSL  KPGSSHRKTK  KPLPPTPEED  QILKKPLPPE  PAAAPVSTSE  LKKVVALYDY
241 MPMNANDLQL  RKGDYFYLE  ESNLPWWRAR  DKNQEGEYIP  SNYVTEAEDS  IEMYEWYSKH
301 MTRSQAQQLL  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  ggcttacgtc  gacgaatggc  cgcagtgatt
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121 aagaagcgcc  tgtttctctt  gaccgtgcac  aaactctcct  actataagta  tgactttgaa
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1921 gtatatacca tcatgtacag ttgttggcat gagaaagcag atgagcgtcc cactttttaa
1981 attcttctga gcaatattct agatgtcatg gatgaagaat cctga
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