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Certificate of Analysis

BTK (R28H), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-765, 14-765-K, 14-765M
Parent Lot # 2067978

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 R28H mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose.
R28H is one of a number of mutations in BTK shown to cause X-linked agammaglobulinemia (XLA), an immune deficiency disorder characterized by a lack of mature, immunoglobulin-producing, peripheral B cells (Ohta *et al*., (1994), Proc. Natl. Acad. Sci. USA, **91**, 9062-9066; Hyvonen & Saraste, (1997), EMBO J. **16**, 3396-3404). Purity 57.3% by SDS-PAGE and Coomassie blue staining. MW = 78.4kDa.

Specific Activity (Parent lot# 2067978): 21U/mg, where one unit of BTK (R28H), active activity is defined as 1nmol phosphate incorporated into 300µM LKBtide (LSNLYHQGKFLQTFCGSPLYRRR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.441mg/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.

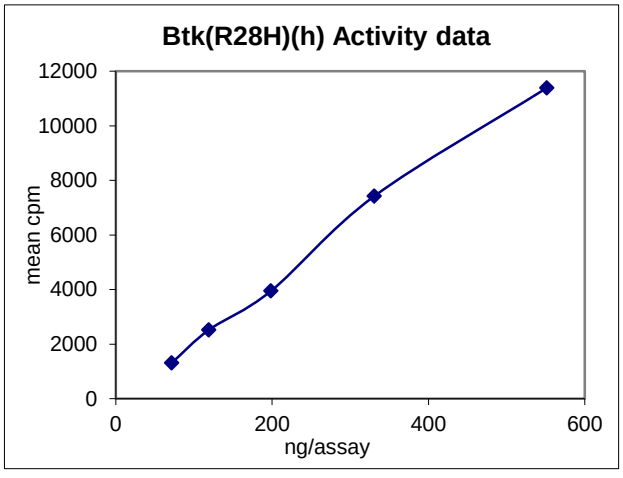
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

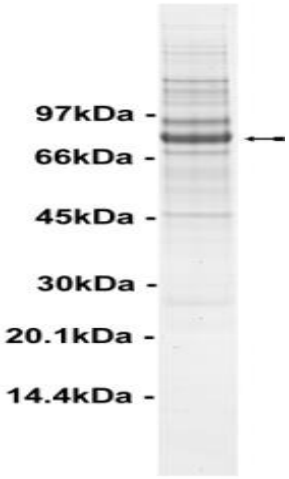
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 25–551ng 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 BTK (R28H), active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom

T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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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 50mM. Make a 3M stock. Add 0.42µl 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 (R28H), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 25–551ng 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 well.
2. Add 2.5µl of **LKBtide**.
3. Add **2.5µl (25–551ng) BTK (R28H), active**.
4. Add 0.42µl of 3M NaCl
5. Add 2.5µl of 10% (w/v) Triton X-100.
6. Make up to 15µl with 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 (R28H) Sequence Information

Protein human BTK (R28H)

Tags *N*-terminal 6His

Native sequence M16 of the recombinant protein is equivalent to M1 of human BTK (R28H)

Accession number GenBank NM_000061

Recombinant BTK amino acid sequence:

```
  1 MHHHHHHEFK GLRRRMAAVI LESIFLKRSQ QKKKTSPLNF KKHLFLLTVH KLSYYEYDFE
 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 aagaagcacc tgtttctctt gaccgtgcac aaactctcct actatgagta tgactttgaa
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 721 atgccaatga atgcaaatga tctacagctg cggaagggtg atgaatattt tatcttggag
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1621 aactgtttgg taaacgatca aggagttgtt aaagtatctg atttcggcct gtccaggtat
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1681 gtcctggatg atgaatacac aagctcagta ggctccaaat ttccagtccg gtggtcccca
1741 ccggaagtcc tgatgtatag caagttcagc agcaaatctg acatttgggc ttttggggtt
1801 ttgatgtggg aaatttactc cctggggaag atgccatatg agagatttac taacagtgag
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1921 gtatatacca tcatgtacag ttgttggcat gagaaagcag atgagcgtcc cacttttaaa
1981 attcttctga gcaatattct agatgtcatg gatgaagaat cctga
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Reviewed and approved by site quality representative.