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Certificate of Analysis

BTK, active
(recombinant enzyme expressed in Sf21 cells)
Cat # 14-552M
Lot # D7HN091U

Product Description: N-terminal 6His-tagged, recombinant, full-length, human BTK, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 82% by SDS PAGE and Coomassie blue staining. MW = 78.4kDa.

Specific Activity (lot# D7HN091U): 356U/mg, where one unit of BTK activity is defined as 1nmol phosphate incorporated into 250µM cdc2 peptide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 250µg of enzyme in 167.9µl of 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

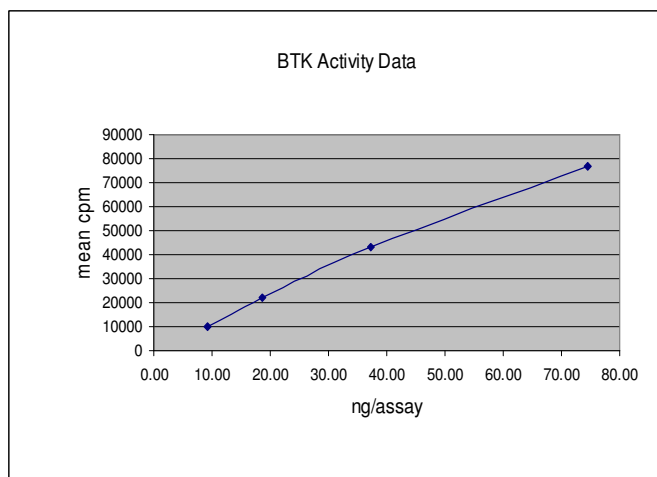
Storage and Stability: Store at -70°C. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

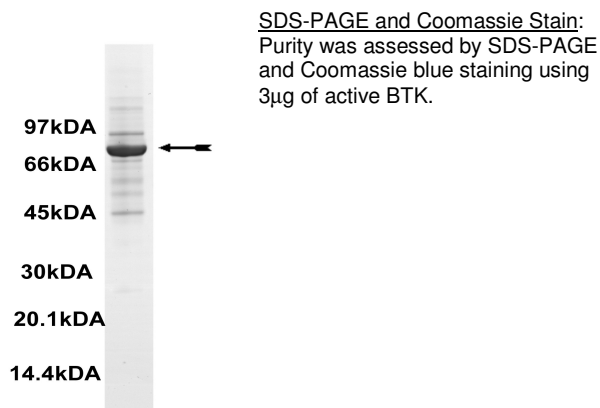
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 9.3-74ng of this lot of enzyme phosphorylated 250µM cdc2 peptide 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 52% amino acid coverage of the translated sequence listed on page three.



Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **cdc2 peptide (KVEKIGEGTYGVVYK):** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **BTK:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 9.3-74ng per assay point.
4. **[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 well.
2. Add 2.5 μ l of cdc2 peptide (KVEKIGEGTYGVVYK).
3. Add **2.5 μ l (9.3-74ng) BTK, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30 °C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 50mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples to cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

BTK Sequence Information

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

Recombinant BTK amino acid sequence:

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1 MHHHHHHEFK GLRRRMAAVI LESIFLKRSQ QKKKTSPLNF KKRLFLLTVH KLSYYEYDFE
61 RGRRGSKKGS IDVEKITCVE TVVPEKNPPP ERQIPRRGEE SSEMEQISII ERFPPYFQVV
121 YDEGPLYVFS PTEELRKRWI HQLKNVIRYN SDLVQKYHPC FWIDGQYLCC SQTAKNAMGC
181 QILENRNGSL KPGSSHRKTK KPLPPTPEED QILKKPLPPE PAAAPVSTSE LKKVVALYDY
241 MPMNANDLQL RKGDEYFILE ESNLPWWRAR DKNQOEGYIP SNYVTEAEDS IEMYEWYSKH
301 MTRSQAEOQLL KQEGKEGGFI VRDSSKAGKY TVSVFAKSTG DPQGVIRHYV VCSTPQSQYY
361 LAEKHLFSTI PELINYHQHN SAGLISRLKY PVSQQKNAP STAGLGYGSW EIDPKDLTFL
421 KELGTGQFGV VKYGKWRGQY DVAIKMIKEG SMSSEDEFIEE AKVMMNLSHE KLVQLYGVCT
481 KQRPIFIITE YMANGCLLNY LREMRHRFQT QQLLEMCKDV CEAMEYLESK QFLHRDLAAR
541 NCLVNDQGVV KVSDFGLSRY VLDDEYTSSV GSKFPVRWSP PEVLMYSKFS SKSDIWAFFGV
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 actatgagta tgactttgaa
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1741 ccggaagtcc tgatgtatag caagttcagc agcaaactctg acatttgggc ttttgggggtt
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1981 attcttctga gcaatattct agatgtcatg gatgaagaat cctga

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