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BTK, active
(Recombinant enzyme expressed in Sf21 insect cells)
Catalogue # 14-552
Lot # 1659960 From bulk lot # D8PN055U

Product Description: N-terminal 6His-tagged, recombinant, full-length, human BTK, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 60.6% by SDS-PAGE and Coomassie blue staining. MW = 78.4kDa.

Specific Activity (lot# 1659960): 369U/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: 10µg of enzyme in 4.9µl 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.

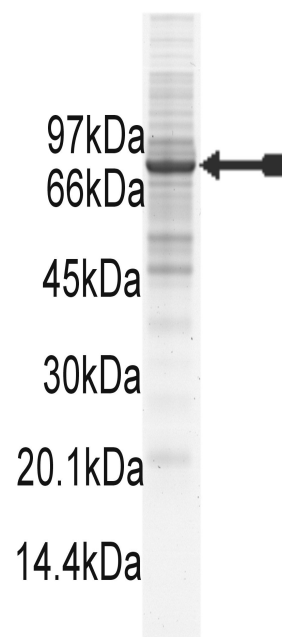
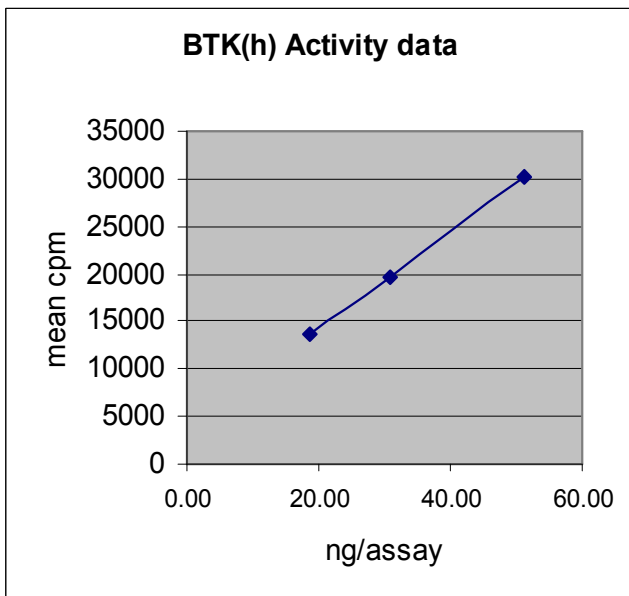
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 19–51ng 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 46% amino acid coverage of 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.



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, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 19–51ng 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 assay to wells.
2. Add 2.5 μ l of **cdc2 peptide (KVEKIGEGTYGVVYK)**.
3. Add **2.5 μ l (19–51ng) 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 with 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
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  KKRLFLLT VH  KLSYYEYDFE
61  RRRRGSKKGS  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 MTRSQAELL  KQEGKEGGFI  VRDSSKAGKY  TVSVFAKSTG  DPQGVIRHYV  VCSTPQSQYY
361 LAEKHLFSTI  PELINYHQHN  SAGLISRLKY  PVSQQNKAP  STAGLGYGSW  EIDPKDLTFL
421 KELGTGQFGV  VKYGKWRGQY  DVAIKMIKEG  SMSDEFIEE  AKVMMNLSHE  KLVQLYGVCT
481 KQRPIFIITE  YMANGCLLNY  LREMRHRFQT  QQLLEMCKDV  CEAMEYLESK  QFLHRDLAAR
541 NCLVNDQGVV  KVSDFGLSRY  VLDDEYTSV  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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121  aagaagcgcc  tgtttctctt  gaccgtgcac  aaactctcct  actatgagta  tgactttgaa
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