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Certificate of Analysis

Blk, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-517, 14-517-K, 14-517M
Parent Lot # 2152754

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 Blk, expressed in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 87.4% by SDS-PAGE and Coomassie blue staining. MW = 58.8kDa.

Specific Activity (Parent lot# 2152754): 152U/mg, where one unit of Blk, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.81mg/ml of enzyme in 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.

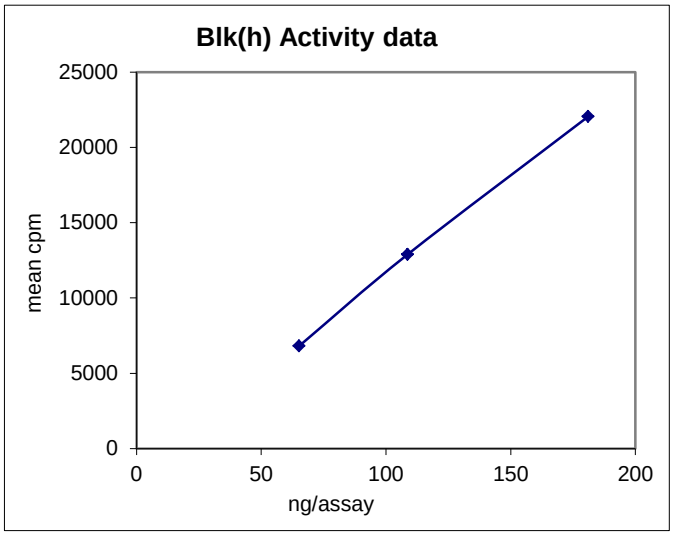
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

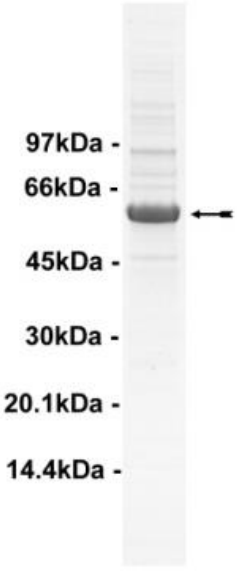
Quality Control Testing

Kinase Assay: 65–181ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) 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 product identity as Blk 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 Blk, active.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na_3VO_4, 1% 2-mercaptoethanol.
2. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock. Use 2.5µl of stock per assay point.
3. **Blk, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na_3VO_4, 0.1% 2-mercaptoethanol. Use 65–181ng per assay point.
4. **[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 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (65–181ng) Blk, active**.
4. Add 7.5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
9. Wash the filtermat three times for 5 minutes with 75mM 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 1µl of 30% phosphoric acid.

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Blk Sequence Information

Protein human Blk

Tags N-terminal 6His

Native sequence M8 of the recombinant protein is equivalent to M1of human Blk

Accession number GenBank NM_001715. The recombinant protein contains one residue which is in conflict with NM_001715. This is M287V and is reported in GenBank S76617 and BC007371.

Recombinant Blk amino acid sequence:

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   1 MHHHHHHMGL VSSKKPDKEK PIKEKDKGQW SPLKVSAQDK DAPPLPPLVV FNHLTPPPPD
  61 EHLDEDKHFV VALYDYTAMN DRDLQMLKGE KLQVLKGTGD WWLARSLVTG REGYVPSNFV
 121 ARVESLEMER WFFRSQGRKE AERQLLAPIN KAGSFLIRES ETNKGAFSLS VKDVTTQGEL
 181 IKHYKIRCLD EGGYYISPRI TFPSLQALVQ HYSKKGDGLC QRLTLPCVRP APQNPWAQDE
 241 WEIPRQSLRL VRKLGSGQFG EVWMGYYKNN MKVAIKTLKE GTMSPEAFLG EANVMKALQH
 301 ERLVRLYAVV TKEPIYIVTE YMARGCLLDF LKTDEGSRLS LPRLIDMSAQ IAEGMAYIER
 361 MNSIHRDLRA ANILVSEALC CKIADFGLAR IIDSEYTAQE GAKFPIKWTA PEAIHFGVFT
 421 IKADVWSFGV LLMEVVTYGR VPYPGMSNPE VIRNLERGYR MPRPDTCPPE LYRGVIAECW
 481 RSRPEERPTF EFLQSVLEDF YTATERQYEL QP
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Recombinant Blk nucleotide sequence:

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   1 atgcatcacc atcaccatca tatggggctg gtaagtagca aaaagccgga caaggaaaag
  61 ccgatcaaag agaaggacaa gggccaatgg agccccctga aggtcagcgc ccaagacaag
 121 gacgccccgc cactgccgcc cctggttgtc ttcaaccacc ttactcctcc accgcccgat
 181 gaacacctgg atgaagacaa gcatttcgtg gtggctctgt atgactacac cgctatgaat
 241 gatcgggacc tgcagatgct gaaggggggag aagctacagg tcctgaaggg aactggagac
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1501 tacacggcca ccgagcggca gtacgagctg cagccctag
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Reviewed and approved by site quality representative.