

Certificate of Analysis

Blk (mouse), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-316, 14-316-K, 14-316M

Parent Lot # D7BN006U

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 mouse Blk, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 88% by SDS-PAGE and Coomassie blue staining. MW = 57.7kDa.

Specific Activity (Parent lot# D7BN006U): 294U/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: 2.012mg/ml of 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 1mM benzamidine, 0.03% Brij-35, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 270mM sucrose. Frozen solution.

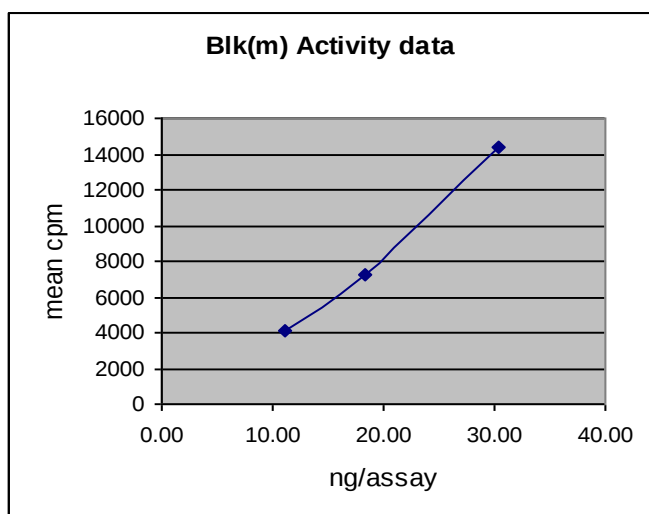
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

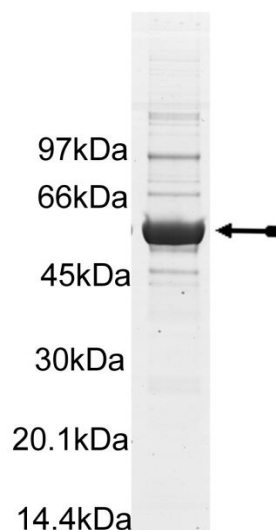
Quality Control Testing

Kinase Assay: 11–30ng 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 identity as mouse 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 (mouse), active.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 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 (mouse), active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol. Use 11–30ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (11–30ng) Blk (mouse),active**.
4. Make up to 15µl with dH₂O.
5. Add 10µl of diluted [γ-³³P]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 (mu) Sequence Information

Protein	mouse Blk
Tags	N-terminal 6His
Native sequence	M8 of the recombinant protein is equivalent to M1 of mouse Blk
Accession number	GenBank M30903. The recombinant protein contains one residue which is in conflict with M30903. This is V421E and is reported in ESTs BF731187 and BG145526.

Recombinant Blk (mu) amino acid sequence:

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1  MHHHHHMHGL LSSKRQVSEK GKGWSPVKIR TQDKAPPLP PLVVFNHLAP PSPNQDPDEE
61  ERFVVALFDY AAVNDRDLQV LKGEKLQVLR STGDWWLARS LVTGREGYVP SNFVAPVETL
121 EVEKWFRTI SRKDAERQLL APMNKAGSFL IRESENKGA FSLSVKDITT QGEVVKHYKI
181 RSLDNGGYI SPRTFPTLQ ALVQHYSKKG DGLCQKLTLP CVNLAPKNLW AQDEWEIPRQ
241 SLKLVRKLGS GQFGEVWMGY YKNNMKVAIK TLKEGTMSPE AFLGEANVMK TLQHERLVRL
301 YAVVTREPIY IVTEYMARGC LLDFLKTDEG SRLSLPRLID MSAQVAEGMA YIERMNSIHR
361 DLRAANILVS ETLCKIADF GLARIIDSEY TAQEGAKFPI KWTAPEAIHF GVFTIKADVW
421 SFGVLLMEIV TYGRVPYPMG SNPEVIRSL EHGVRMPCPET CPPELYNDII TECWRGRPEE
481 RPTFEFLQSV LEDFYTATEG QYELQP

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Recombinant Blk (mu) nucleotide sequence:

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1  atgcatcatc accatcacca tatggggctg ctgagcagca agaggcaggt cagtgagaag
61  ggcaagggtt ggagccccgt gaagatccgc acccaggaca aggctcccc acccctgcca
121 cccttggttg tcttaacca ccttgcccca ccattctcta accaggacc agatgaagag
181 gagcgttttg tgggtgctct gtttgactat gccgctgtga atgacaggga ccttcagggtg
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1501 caatatgagc tgcagcccta g

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