

# Blk Kinase

✓ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant full length human Blk (Met1-Pro505) kinase, supplied as a GST fusion protein.

**Background:** Blk is a Src family protein tyrosine kinase expressed in all stages of B cell development (1,2). Activation of B cells by various ligands is accompanied by activation of Blk (3). It has been suggested that Blk is involved in the control of B cell differentiation and proliferation (4,5). Blk transcripts have also been detected in human thymocytes, but not in mature T cells, implicating that Blk may play an important role in thymopoiesis (6). Blk function may be redundant, however, as mice that do not express Blk are not impaired with respect to B cell development and immune response (7).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human Blk (Met1-Pro505) (GenBank Accession No. BC007371) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

**Quality Control:** The theoretical molecular weight of the GST-Blk fusion protein is 84 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Blk kinase activity was determined using a radiometric assay [Fig.2].

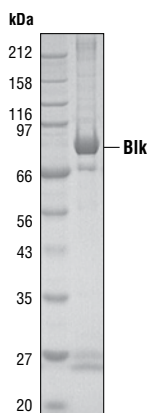


Figure 1. The purity of the GST-Blk fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

## Background References:

- (1) Dymecki, S.M. et al. (1990) *Science* 247, 332–336.
- (2) Wasserman, R. et al. (1995) *J. Immunol.* 155, 644–651.
- (3) Sefton, B.M. et al. (1994) *Curr. Opin. Immunol.* 6, 372–379.
- (4) Malek, S.N. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 7351–7356.
- (5) Yao, X.R. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 7946–7950.
- (6) Islam, K.B. et al. (1995) *J. Immunol.* 154, 1265–1272.
- (7) Texido, G. et al. (2000) *Mol. Cell Biol.* 20, 1227–1233.

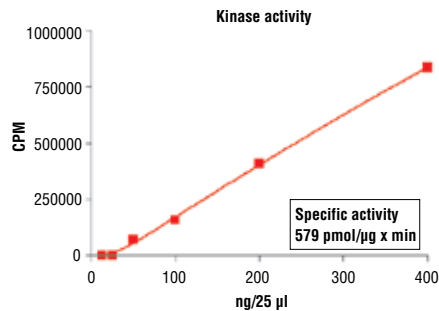


Figure 2. Blk kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM  $\beta$ -glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 10 mM  $MnCl_2$ , 4 mM  $MgCl_2$ , 0.05 mM DTT, 50  $\mu$ M ATP. Substrate: Poly(Glu-Tyr), 400 ng/ $\mu$ L and recombinant Blk: variable.

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at  $-80^\circ\text{C}$ .

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

## Protocol for Blk Kinase Assay

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

### A Additional Solutions and Reagents (Not included)

- 1. Kinase Buffer (10X)**  
40 mM MOPS, pH 7.2  
25 mM  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
100 mM  $MgCl_2$   
40 mM  $MgCl_2$   
0.5 mM DTT
- 2. ATP (10 mM) #9804**
- 3.  $^{32}P$ - $\gamma$ ATP**
- 4. Poly(Glu-Tyr) (1  $\mu$ g/ $\mu$ l)**

### B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
- 2.** Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute Blk kinase protein (100 ng/ $\mu$ l concentration) to 20 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10  $\mu$ l diluted Blk kinase solution, 10  $\mu$ l Poly(Glu-Tyr) (1  $\mu$ g/ $\mu$ l) and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

### Final Assay Conditions

- 4 mM MOPS, pH 7.2
  - 2.5 mM  $\beta$ -glycerophosphate
  - 1 mM EGTA
  - 0.4 mM EDTA
  - 10 mM  $MnCl_2$
  - 4 mM  $MgCl_2$
  - 0.05 mM DTT
  - 400 ng/ $\mu$ l Poly(Glu-Tyr)
- 6.** After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
  - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
  - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
  - 9.** Count samples in a scintillation counter.

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