BLK, Active
human, recombinant
GST-tagged, expressed in Sf9 cells

Catalog Number B8811
Lot Number 019K1561
Storage Temperature –70 °C

Synonym: MGC10442

Product Description
BLK, also known as B lymphoid kinase, is a 55 kDa tyrosine kinase with SH3, SH2, and catalytic domains that contain consensus sequences of the src protein tyrosine kinase family. BLK is expressed specifically in the B cell lineage and plays a role in the signal transduction pathway that is restricted to B lymphoid cells. Stimulation of resting B lymphocytes with antibodies to surface immunoglobulin (slgD or slgM) induces activation of BLK.

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 009594. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~84 kDa
Purity: ≥70% (SDS-PAGE, see Figure 1)
Specific Activity: 95–129 nmole/min/mg (see Figure 2)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Procedure
Preparation Instructions
Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 20 mM MgCl2, 25 mM MnCl2, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with water.
Kinase Solution – Dilute the Active BLK (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. **Note:** The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of Active BLK kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

γ-32P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-32P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate Poly (Glu:Tyr, 4:1) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

**Kinase Assay**

This assay involves the use of the 32P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the Active BLK, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-32P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
   - 10 µl of Kinase Solution
   - 10 µl of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-32P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of –10 minutes each.
7. Set up a radioactive control to measure the total γ-32P-ATP counts introduced into the reaction. Spot 5 µl of the γ-32P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

**Calculations:**

1. Specific Radioactivity (SR) of ATP (cpm/nmole)
   \[ SR = \frac{\text{cpm of } 5 \mu l}{\text{nmole of ATP}} \]
   \[ \text{nmole of ATP} = 1.25 \text{ nmole (5 } \mu l \text{ of 250 } \mu M \text{ ATP Assay Cocktail)} \]

2. Specific Kinase Activity (SA) (nmole/min/mg)
   \[ \text{nmole/min/mg} = \frac{\Delta \text{cpm} \times (25/20)}{SR \times E \times T} \]
   \[ 25 = \text{total reaction volume} \]
   \[ 20 = \text{spot volume} \]
   \[ T = \text{reaction time (minutes)} \]
   \[ E = \text{amount of enzyme (mg)} \]

**References**


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