Recombinant Human Active Blk Protein, CF

Certificate of Analysis

Catalog Number: 2679-KS-010

Lot Number: 1655678

SPECIFICATIONS AND USE

Source	Recombinant human Blk was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is BC007371.
Molecular Mass	The approximate molecular weight is 84 kDa.
Purity	The purity was determined to be > 90% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 10 mM Glutathione, 0.1 mM EDTA, 0.1 mM PMSF, and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of Blk was determined to be 110 nmol/min/mg using a poly (4:1 Glu, Tyr) synthetic peptide substrate.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

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 signal transduction pathway that is restricted to B lymphoid cells (1). Stimulation of resting B lymphocytes with antibodies to surface immunoglobulin (sIgD or sIgM) induces activation of BLK (2).

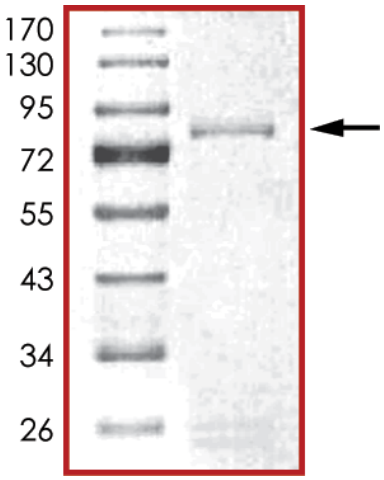

Figure 1: The purity of Blk was determined to be > 90% by densitometry. Approximately MW 88kDa.

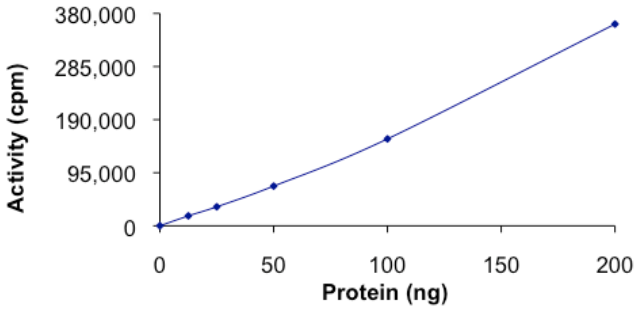

Figure 2: Enzymatic assay results. The specific activity of Blk was determined to be 110 nmol/min/mg as per activity assay protocol (on reverse).

REFERENCES

1. Dymecki, S.M. *et al.* (1990) Science **247**:332.
2. Burkhardt, A.L. *et al.* (1991) Proc. Natl. Acad. Sci. USA **88**:7410.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active Blk (0.1 µg/µL) diluted with Kinase Dilution Buffer II and assayed as outlined in sample activity plot.
Note: *These are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active Blk for optimal results.*

Kinase Dilution Buffer II - Kinase Assay Buffer II diluted at a 1:4 ratio (5X dilution) with distilled water.

Kinase Assay Buffer II - 25 mM MOPS, pH 7.2, 12.5 mM β glycerol-phosphate, 20 mM $MgC1_2$, 25 mM $MnCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to Kinase Assay Buffer prior to use

[^{33}P]-ATP Assay Cocktail - Prepare 250 µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 µL of 10 mM ATP Stock Solution, 100 µl [^{33}P]-ATP (1 mCi/100 µL), 5.75 mL of Kinase Assay Buffer II. Store 1 mL aliquots at -20 °C.

10 mM ATP Stock Solution - Prepare ATP stock solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer II. Store 200 µL aliquots at -20 °C.

Substrate - Poly (4:1 Glu, Tyr) synthetic peptide substrate diluted in distilled water to a final concentration of 1 mg/mL.

ASSAY PROCEDURE

1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
2. Thaw the Active Blk, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL:

Reaction Component	Amount
Diluted Active Blk	10 µL
Stock solution of Substrate at 1 mg/mL	5 µL
Distilled water (2-8 °C)	5 µL

4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled water.
5. Initiate the reaction by the addition of 5 µL [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25 µL and incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid and make a 1 L solution with distilled water) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)
Specific activity (SA) = cpm for 5 µL [^{33}P]-ATP / pmoles of ATP (in 5 µL of a 250 µM ATP stock solution, *i.e.,* 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)
Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)] * [(Reaction Volume) / (Spot Volume)]

H. Helen Zhang
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