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Product Specification

BLK, active
(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7723
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 579 nmol/min/mg

Quality Control Analysis
Activity assessment
BLK protein 100 ng/µl concentration) was diluted to 20ng/µl in assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 10 mM MnCl2, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Poly(Glu-Tyr) in the following assay condition:

10 µl Diluted BLK protein
5 µl Poly(Glu-Tyr) (1 mg/ml stock)
5 µl water
5 µl [^{32}P] ATP (250 µM ATP stock, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30° C and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

Fig. 1 BLK activity assay

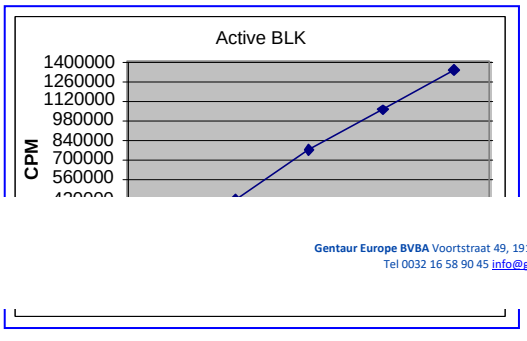
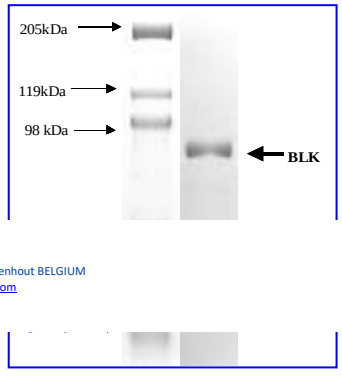


Fig. 2 BLK protein gel



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Purity assessment
1 µg of BLK protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the BLK product, and the band was at ~84 kDa (Fig. 2).

Product Description
Recombinant full-length human BLK containing N-terminal GST tag expressed by baculovirus in Sf 9 insect cells.
The gene accession number is BC007371.
This material is sold for research purposes only.

Specific Activity
579 nmol phosphate incorporated into Poly(Glu-Tyr) per minute per mg protein at 30° C using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation
Recombinant protein in storage buffer (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).

Storage and Stability
Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background
Blk, also known as B lymphoid kinase, is a 55 kd tyrosine kinases with a 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). Upon activation, the SH2 domain of Blk binds more than 10 distinct phosphoprotein including those of 72, 76, 115, and 130 kd (3). Blk prefers peptide substrates of the structure I/L-Y-D/E-X-L which resemble critical features of the ITAM motifs found in, e.g. the intracellular components Ig-alpha and Ig-beta of the beta cell receptor. Blk has a strong preference for a negatively charged amino acid in position +1 (4).

References
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3. Malek SN, Desiderio S. SH2 domains of the protein-tyrosine kinases Blk, Lyn, and Fyn(T) bind distinct sets of phosphoproteins from B lymphocytes. J Biol Chem. 1993 Oct 25;268(30):22557-65.
4. Schmitz R, Baumann G, Gram H. Catalytic specificity of phosphotyrosine kinases Blk, Lyn, c-Src and Syk as assessed by phage display. J Mol Biol. 1996 Aug 2;260(5):664-77.

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