

## Product Specification

### **BMX, active**

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

Catalog #: 7732  
 Lot #: \_\_\_\_\_  
 Aliquot size: 5µg protein in 50 µl  
 Specific activity: 29 nmol/min/mg

### **Quality Control Analysis**

#### Activity assessment

BMX 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, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl<sub>2</sub>, 0.05 mM DTT and 40ng/ul BSA), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Poly Glu-Tyr substrate in the following assay condition:

- 10 µl diluted BMX protein
- 10 µl Poly Glu-Tyr substrate (1 mg/ml stock)
- 5 µl [<sup>32</sup>P] ATP (250 µM ATP stock, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [<sup>32</sup>P] ATP, were incubated at 30<sup>0</sup>C and the reaction started by the addition of [<sup>32</sup>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.

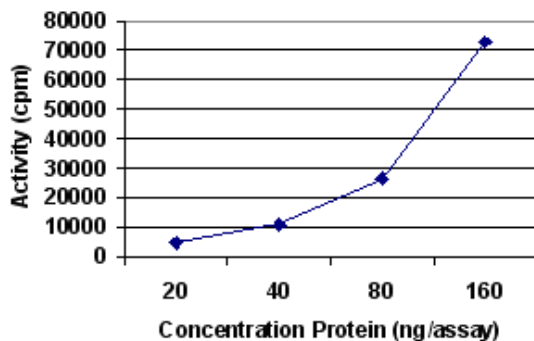


Fig. 1 BMX activity assay

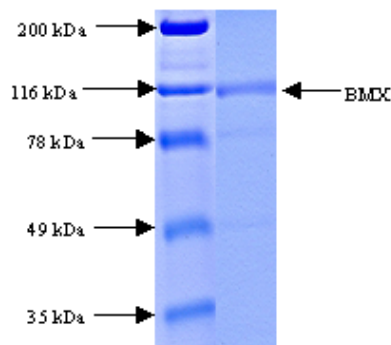


Fig. 2 BMX protein gel

#### Purity assessment

1 µg of BMX protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >80% purity of the BMX product, and the major band was at ~110 kDa (Fig. 2).

### **Product Description**

Recombinant full length human BMX containing N-terminal GST-tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM\_001721.

This material is sold for research purposes only.

### **Specific Activity**

29 nmol phosphate incorporated into Poly Glu-Tyr substrate 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**

The BMX gene encodes a novel non receptor tyrosine kinase, which may play a role in the growth and differentiation of hematopoietic cells (1). Bmx cDNA comprises a long open reading frame of 675 amino acids, containing one SH3, one SH2 and one tyrosine kinase domain, which are about 70% identical with Btk, Itk and Tec and somewhat less with Txk tyrosine kinase sequences. The amino terminal sequences of these four tyrosine kinases are about 40% identical and each contains a so-called pleckstrin homology (PH) domain. The 2.7 kb Bmx mRNA is expressed in endothelial cells and several human tissues by Northern blotting and an 80 kD Bmx polypeptide was detected in human endothelial cells. The BMX gene is located in chromosomal band Xp22.2 between the DXS197 and DXS207 loci. Interestingly, chromosome X also contains the closest relative of BMX, the BTK gene, implicated in X-linked agammaglobulinemia. Bmx, is found to induce activation of the Stat signaling pathway (3). Bmx induced the tyrosine phosphorylation and DNA binding activity of all the Stat factors tested, including Stat1, Stat3, and Stat5, both in mammalian and insect cells. Bmx also induced transcriptional activation of Stat1- and Stat5-dependent reporter genes.

### **References**

1. Tamagnone L, Lahtinen I, Mustonen T, Virtaneva K, Francis F, Muscatelli F, Alitalo R, Smith CI, Larsson C, Alitalo K. BMX, a novel nonreceptor tyrosine kinase gene of the BTK/ITK/TEC/TXK family located in chromosome Xp22.2. *Oncogene*. 1994 Dec;9(12):3683-8.
2. Kaukonen J, Lahtinen I, Laine S, Alitalo K, Palotie A. BMX tyrosine kinase gene is expressed in granulocytes and myeloid leukaemias. *Br J Haematol*. 1996 Sep;94(3):455-60.
3. Saharinen P, Ekman N, Sarvas K, Parker P, Alitalo K, Silvennoinen O. The Bmx tyrosine kinase induces activation of the Stat signaling pathway, which is specifically inhibited by protein kinase Cdelta. *Blood*. 1997 Dec 1;90(11):4341-53.