570-5600 Parkwood Way
Richmond, BC V6V2M2
CANADA
Tel: 1-(604)-232-4600
Fax: 1-(604)-232-4601
orders@signalchem.com

BMX, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # B07-10G
Lot # A247-1

Product Description

Recombinant full-length human BMX was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_001721.

Gene Aliases

ETK; PSCTK2; PSCTK3

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

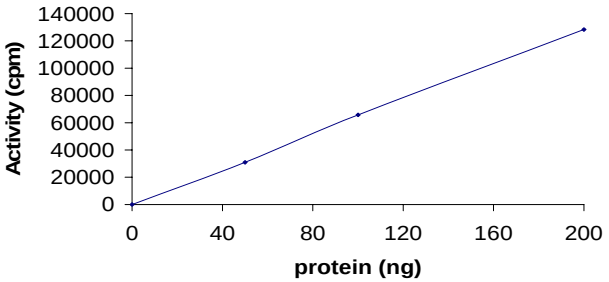
Scientific Background

The BMX gene encodes a non-receptor tyrosine kinase, which may play a role in the growth and differentiation of hematopoietic cells (1). The BMX gene is located on 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 (2).

References

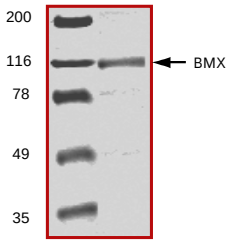
1. Tamagnone, L. et al: BMX, a novel nonreceptor tyrosine kinase gene of the BTK/ITK/TEC/TXK family located in chromosome Xp22.2. Oncogene. 1994 Dec;9(12):3683-8.
2. Saharinen, P. et al: The Bmx tyrosine kinase induces activation of the Stat signaling pathway, which is specifically inhibited by protein kinase Cdelta. Blood. 1997 Dec 1;90(11):4341-53

Specific Activity



The specific activity of BMX was determined to be **33 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>80%** by densitometry.
Approx. MW **110kDa**.

BMX, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	B07-10G-10
Quantity	10µg (5µg x 2)
Specific Activity	33 nmol/min/mg
Specific Lot Number	A247-1
Purity	>80%
Format	(5µg in 50µl) x 2
Concentration	0.1µg/µl
Stability	1yr At –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: B07-10G-10)

Active BMX (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K28-09) 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 BMX for optimal results).

Kinase Dilution Buffer, pH 7.2 (Catalog #: K28-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution containing 5% glycerol.

Kinase Assay Buffer II, pH 7.2 (Catalog #: K02-09)

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 25mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use

[^{32}P]-ATP Assay Cocktail

Prepare 250µM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^{32}P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K02-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K02-09). Store 200µl aliquots at –20°C.

Substrate

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active BMX, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 10µl of diluted Active BMX (Catalog #B07-10G-10).
- **Component 2.** 10µl of 1mg/ml stock solution of substrate

Step 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 H_2O.

Step 5. Initiate the reaction by the addition of 5µl [^{32}P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 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 [P^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [^{32}P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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MATERIAL SAFETY DATA SHEET

Article 1 - Product Identification and Use

Product Name: BMX, Active **Catalog # B07-10G-10**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name:	SignalChem Pharmaceuticals Inc.
Street Address:	*570-5600 Parkwood Way*
City, Prov. Postal Code:	*Richmond, BC, V6V 2M2*
Fax:	604-232-4601
EMERGENCY PHONE:	604-232-4600

Article 2 - Hazardous Ingredients

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

Article 3 - Physical Data

This product consists of purified protein in Tris-HCl buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

Article 4 - Fire and Explosion Hazard

NOT APPLICABLE

Article 5 - Reactivity Data

NOT APPLICABLE

Article 6 – Toxicologically Data

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

Article 7 - Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

*****MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES*****

- Wear protective equipment.
- Absorb on sand or vermiculite and place in closed containers for disposal.
- Observe all federal, state and local environmental regulations.

Article 8 - First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
- If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
- In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.

Article 9 - Preparation

Prepared By: Phone #: