

Etk/BMX Kinase

✓ 5 µg



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full length human Etk/BMX (Met1-His675) kinase, supplied as a GST fusion protein.

Background: Etk, also known as BMX, is a member of the Bruton's tyrosine kinase (Btk) family (1). It is expressed in a variety of hematopoietic, epithelial and endothelial cells. Etk, like other Btk family members, contains a pleckstrin homology (PH) domain and Src homology SH3 and SH2 domains. It participates in multiple signal transduction pathways (2). Phosphorylation of Tyr566 by Src kinase is required for activation of Etk *in vivo* (3). In endothelial and epithelial cells, Etk is regulated by FAK through phosphorylation at Tyr40 (4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human Etk/BMX (Met1-His675) (GenBank Accession No. NM_001721) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

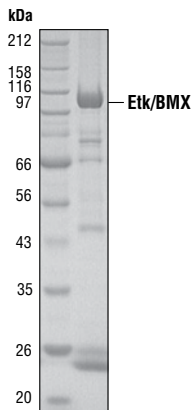


Figure 1. The purity of the GST-Etk/BMX fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-Etk/BMX fusion protein is 104 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Etk/BMX kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Tamagnone, L. et al. (1994) *Oncogene* 9, 3683–3688.
- (2) Qiu, Y. and Kung, H.J. (2000) *Oncogene* 19, 5651–5661.
- (3) Tsai, Y.T. et al. (2000) *Mol. Cell. Biol.* 20, 2043–2054.
- (4) Chen, R. et al. (2001) *Nat. Cell Biol.* 3, 439–444.

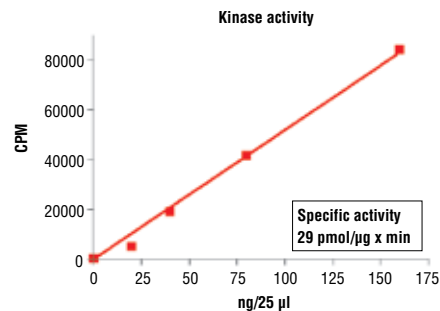


Figure 2. Etk/BMX kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 10 mM MnCl₂, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT, 40 ng/µL BSA, 50 µM ATP, Substrate: Poly(Glu-Tyr), 400 ng/µL and recombinant Etk/BMX: variable.

Storage: Enzyme is supplied in 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, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Protocol for Etk/BMX Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. Kinase Buffer (10X)

40 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM EDTA
40 mM $MgCl_2$
100 mM $MnCl_2$
0.5 mM DTT
400 ng/ μ l BSA

2. ATP (10 mM) #9804

3. ^{32}P - γ ATP

4. Poly(Glu-Tyr) (1 μ g/ μ l)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
2. Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
3. Transfer enzyme from $-80^\circ C$ to ice. Allow enzyme to thaw on ice.
4. Dilute Etk/BMX kinase protein (100 ng/ μ l concentration) to 20 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 μ l diluted Etk/BMX kinase solution, 10 μ l Poly(Glu-Tyr) (1 μ g/ μ l) and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 4 mM MOPS, pH 7.2
2.5 mM β -glycerophosphate
1 mM EGTA
0.4 mM EDTA
4 mM $MgCl_2$
10 mM $MnCl_2$
0.05 mM DTT
40 ng/ μ l BSA
400 ng/ μ l Poly(Glu-Tyr)
6. After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
 7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 9. Count samples in a scintillation counter.

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