Store at –80°C

#7377

Btk Kinase

☑5 µg

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New 05/07

Description: Purified recombinant full-length human Btk (Met1-Ser659) kinase, supplied as a protein with amino-terminal His tag.

Background: Bruton+s tyrosine kinase (Btk) is a member of the Btk/Tec family of cytoplasmic tyrosine kinases. Like other Btk family members, it contains a pleckstrin homology (PH) domain, and Src homology SH3 and SH2 domains. Btk plays an important role in B cell development (1,2). Activation of B cells by various ligands is accompanied by Btk membrane translocation mediated by its PH domain binding to phosphatidylinositol-3,4,5-trisphosphate (3-5). The membrane-located Btk is active and associated with transient phosphorylation of two tyrosine residues, Tyr551 and Tyr223. Tyr551 in the activation loop is transphosphorylated by the Src family tyrosine kinase, leading to autophosphorylation at Tyr223 within the SH3 domain, which is necessary for full activation (6,7). The activation of Btk is negatively regulated by PKCβ through phosphorylation of Btk at Ser180, which results in reduced membrane recruitment, transphosphorylation and subsequent activation (8). The PKC inhibitory signal is likely to be a key determinant of the B-cell receptor signaling threshold to maintain optimal Btk activity (8).

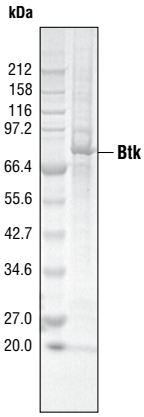

Figure 1. The purity of the Btk fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The His tag protein was produced using a baculovirus expression system with a construct expressing full-length human Btk (Met1-Ser659) (GenBank Accession No. NM_000061) with an amino-terminal His tag. The protein was purified by Immobilized Metal Affinity Chromatography (IMAC).

Quality Control: The theoretical molecular weight of the Btk fusion protein is 78 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. Btk kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Khan, W.N. (2001) *Immunol. Res.* 23, 147–156.

(2) Lewis, C.M. et al. (2001) *Curr. Opin. Immunol.* 13, 317–325.

(3) Salim, K. et al. (1996) *EMBO J.* 15, 6241–6250.

(4) Rameh, L.E. et al. (1997) *J. Biol. Chem.* 272, 22059–22066.

(5) Varnai, P. et al. (1999) *J. Biol. Chem.* 274, 10983–10989.

(6) Rawlings, D.J. et al. (1996) *Science* 271, 822–825.

(7) Park, H. et al. (1996) *Immunity* 4, 515–525.

(8) Kang, S.W. et al. (2001) *EMBO J.* 20, 5692–5702.

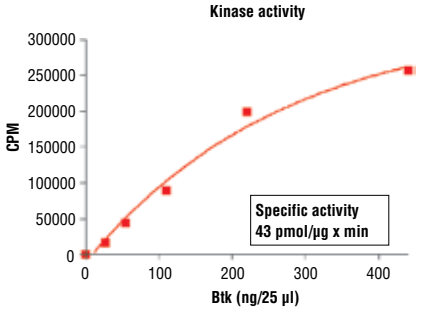

Figure 2. Btk kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: Poly EY (E:Y, 4:1) 200 ng/µL, and recombinant Btk: variable.

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.

Companion Products:
HTScan® Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

Tyrosine Kinase Substrate Screening Kit #7450

Protocol for Btk 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)**
   50 mM MOPS, pH 7.2
   25 mM β-glycerophosphate
   10 mM EGTA
   4 mM EDTA
   40 mM $MgC1_2$
   0.5 mM DTT
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. Poly EY (E:Y, 4:1, 0.5 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute Btk protein to 40 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted Btk kinase solution, 10 µl Poly EY (0.5 µg/µl), and 5 µl 0.16 µCi/µl [^{32}p] ATP solution.

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
0.4 mM EDTA
4 mM $MgCl_2$
0.05 mM DTT
400 ng/µl Poly EY

6. After 15 minutes terminate reaction by spotting 20 µl 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.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.