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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
B10-12CH-05	5 µg
B10-12CH-10	10 µg

BTK (C481S), Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog # B10-12CH

Lot # L2208-9

Product Description

Recombinant full-length human BTK (C481S) was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_000061.

Gene Aliases

AT; ATK; BPK; XLA; IMD1; AGMX1; PSCTK

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, and 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

BTK (also known as Bruton tyrosine kinase) plays a crucial role in B-lymphocyte differentiation and activation. BTK interacts with SRC homology 3 domains of FYN, LYN and HCK that are activated upon stimulation of B- and T-cell receptors (1).Defects in the BTK gene cause Agammaglobulinemia, an X-linked immunodeficiency characterized by failure to produce mature B lymphocyte cells and associated with a failure of Ig heavy chain rearrangement. The unique role of BTK makes it a desirable target for potential anti-cancer, anti-inflammatory and anti-viral agents as well as other treatments (2).

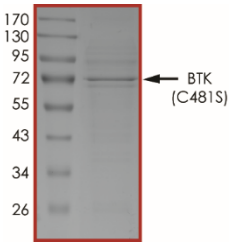
References

1. Cheng, G. et al: Binding of Bruton's tyrosine kinase to Fyn, Lyn, or Hck through a Src homology 3 domain-mediated interaction. *Proc. Nat. Acad. Sci.* 91: 8152-8155, 1994.
2. Vassilev, A O. et al: Therapeutic potential of inhibiting Bruton's tyrosine kinase, (BTK).Curr Pharm Des. 2004;10(15):1757-66.

Specific Activity

The specific activity of BTK (C481S) was determined to be **65 nmol /min/mg** as per activity assay protocol.

Purity



The purity of BTK (C481S) was determined to be **>70%** by densitometry, approx. MW **~75kDa**.

BTK (C481S), Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog #	B10-12CH
Specific Activity	65 nmol/min/mg
Lot #	L2208-9
Purity	>70%
Concentration	0.1 µg/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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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: B10-12CH)

Active BTK (C481S) (0.1μg/μl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 BTK (C481S) for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}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 [^{33P}]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). 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 I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: P61-58)

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

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active BTK (C481S), Kinase Assay Buffer, Substrate and Kinase 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 BTK (C481S) (Catalog #B10-12CH)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #P61-58)
- **Component 3.** 5μl distilled H_2O (4°C)

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 5 μl [^{33}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 20 μl 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^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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