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## Certificate of Analysis

### Anti-BTK

(mouse ascites)

Catalog # 05-415

Lot # 23098

**Immunogen:** Partial fusion protein corresponding to residues 1-175 of human BTK. Clone 10D11.

**Specificity:** Recognizes human BTK, Mr 77kDa. A higher molecular weight protein may be detected, which is thought to be a phosphorylated species of BTK or a related protein.

**Species Cross-reactivity:** Human. Other species not tested.

**Formulation:** 200µl of mouse ascites containing 0.02% sodium azide. Frozen solution.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

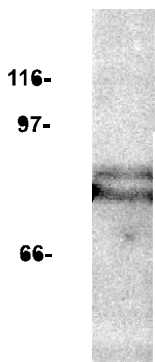
**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

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### Quality Control Testing

**Immunoblot Analysis:** A 1:1000-1:2000 dilution of this lot detected BTK in RIPA lysates from Raji cells.

**Immunocytochemistry:** Not recommended.



#### Immunoblot Analysis

Representative blot from a previous lot. Raji cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-BTK (1:1000 dilution). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates BTK (77kDa).

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### Additional Research Applications

**Immunoprecipitation:** This antibody has been reported by an independent laboratory to immunoprecipitate BTK in the presence of 0.5% SDS. Not recommended for IP/kinase assays.

**Chromatin Immunoprecipitation:** Reported by an independent laboratory.

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### General References:

1. Tianjian, L. *et al.*, *Immunity* **2**: 451-460, 1995.
2. Bykowsky, M.J. *et al.*, *Am. J. Hum. Genet.* **58**: 477-483, 1996.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with a **1:1000-1:2000 dilution of anti-BTK**, diluted in freshly prepared PBS-MLK, overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-mouse** HRP conjugated IgG, Catalog # 12-349, 1:2000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween<sup>®</sup>-20 for 15 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).