

Anti-Human BTK

(mouse ascites IgG₁)

Catalog # 05-415

Lot # 15722

Immunogen: Partial fusion protein corresponding to residues 1-175 of human BTK.

Specificity: Recognizes human BTK at 77kDa.

Species Cross-reactivity: Not tested.

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.

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

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

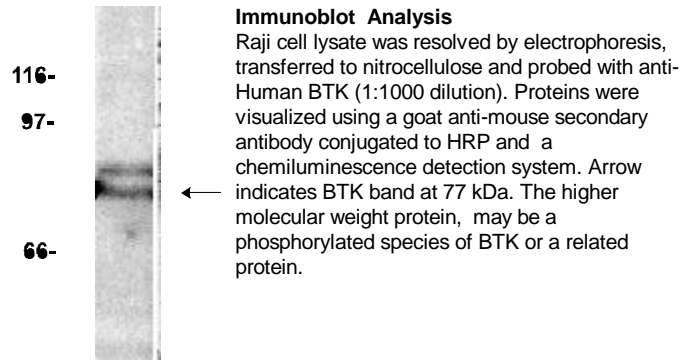
Quality Control Testing

Immunoblot Analysis: A 1:1000 dilution of this lot detected BTK in RIPA lysates from Raji cells. A higher molecular weight protein may also be detected, which may be a phosphorylated species of BTK or a related protein.

Immunocytochemistry: Not recommended.

Additional Research Applications

Immunoprecipitation: This antibody is reported to immunoprecipitate BTK in the presence of 0.5% SDS. Not recommended for IP/kinase assays.



Background:

Bruton's tyrosine kinase (BTK) is a cytoplasmic non-receptor tyrosine kinase that is expressed in most hematopoietic tissues. BTK belongs to a new subfamily of non-receptor tyrosine kinases which includes Tec I, Tec II, Itk, and Dsrc28C. The structure of non-receptor tyrosine kinases is characterized by the Src homology (SH) tyrosine kinase domains including SH2 and SH3. The most distinctive feature of the BTK family of tyrosine kinases is the presence of a pleckstrin homology domain in its amino-terminal region. BTK is critical for B cell development and function, and was recently identified as the defective gene in human X-linked agammaglobulinemia and murine X-linked immunodeficiency.

General References:

Tianjian, L, *et al.*, Immunity **2**: 451-460, 1995.

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 EGTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 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 (PBS-MLK) for 30 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1:1000 dilution of α-Human 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, 1:3000 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 20 for 15 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).