

**bs-3069R****[ Primary Antibody ]****phospho-BTK (Tyr223) Rabbit pAb****BioSS**  
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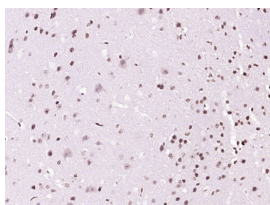
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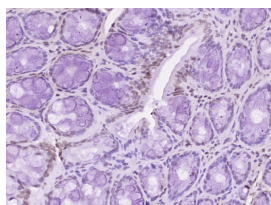
**— DATASHEET —****Host:** Rabbit**Isotype:** IgG**Clonality:** Polyclonal**GeneID:** 695**SWISS:** Q06187**Target:** BTK (Tyr223)**Immunogen:** KLH conjugated Synthesised phosphopeptide derived from human Btk around the phosphorylation site of Tyr223: AL(p-Y)DY.**Purification:** affinity purified by Protein A**Concentration:** 1mg/ml**Storage:** 0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol.

Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.

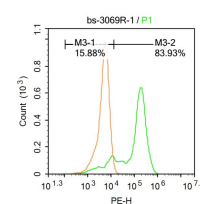
**Background:** Brutons tyrosine kinase (BTK) is a member of the BTK/Tec family of cytoplasmic tyrosine kinases. Like other BTK family members, it contains a pleckstrin homology (PH) domain, Src homology SH3 and SH2 domains. BTK plays an important role in B cell development. Activation of B cells by various ligands is accompanied by BTK membrane translocation mediated by its PH domain binding to phosphatidylinositol-3,4,5-trisphosphate. 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. The activation of BTK is negatively regulated by PKC beta through phosphorylation of BTK at Ser180, which results in reduced membrane recruitment, transphosphorylation and subsequent activation. The PKC/BTK inhibitory signal is likely to be a key determinant of the B cell receptor signaling threshold to maintain optimal BTK activity.

**Applications:** IHC-P (1:100-500)**IHC-F** (1:100-500)**IF** (1:100-500)**Flow-Cyt** (1ug/test)**ELISA** (1:5000-10000)**Reactivity:** Human, Mouse, Rabbit  
(predicted: Rat, Pig, Cow, Horse)**Predicted MW.:** 76 kDa**Subcellular Location:** Cell membrane ,Cytoplasm ,Nucleus**— VALIDATION IMAGES —**

Paraformaldehyde-fixed, paraffin embedded (Mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-BTK (Tyr223)) Polyclonal Antibody, Unconjugated (bs-3069R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (Mouse colon); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-BTK (Tyr223)) Polyclonal Antibody, Unconjugated (bs-3069R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.



U-937 cells were fixed with 4% PFA for 10min at room temperature, permeabilized with 90% ice-cold methanol for 20 min at room temperature, and incubated in 5% BSA blocking buffer for 30 min at room temperature. Cells were then stained with Phospho-BTK (Tyr223) Antibody(bs-3069R) at 1:100 dilution in blocking buffer and incubated for 30 min at room temperature, washed twice with 2%BSA in PBS, followed by secondary antibody incubation for 40 min at room temperature. Acquisitions of 20,000 events were performed. Cells stained with primary antibody (green), and isotype control (orange).

## — SELECTED CITATIONS —

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- **[IF=6.575]** Gengguo Deng. et al. Ibrutinib Inhibits BTK Signaling in Tumor-Infiltrated B Cells and Amplifies Antitumor Immunity by PD-1 Checkpoint Blockade for Metastatic Prostate Cancer. *CANCERS*. 2023 Jan;15(8):2356 IF,IHC ;Mouse,Human. 37190284