Revision 1

#5082 Store at -20C

Phospho-Btk (Tyr223) Antibody

Cell Signaling TECHNOLOGY®

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB	H	Endogenous	77	Rabbit	#Q06187	695

Product Usage Information

Application	Dilution
Western Blotting	1:1000

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

Phospho-Btk (Tyr223) Antibody detects endogenous levels of Btk only when phosphorylated at Tyr223.

Species predicted to react based on 100% sequence homology:

Mouse, Rat

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr223 of human Btk protein. Antibodies are purified by protein A and peptide affinity chromatography.

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-localized 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 kinases, 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).

Background References

1. Khan, W.N. (2001) *Immunol Res* 23, 147-56.
2. Lewis, C.M. et al. (2001) *Curr Opin Immunol* 13, 317-25.
3. Salim, K. et al. (1996) *EMBO J* 15, 6241-50.
4. Rameh, L.E. et al. (1997) *J Biol Chem* 272, 22059-66.
5. Várnai, P. et al. (1999) *J Biol Chem* 274, 10983-9.
6. Rawlings, D.J. et al. (1996) *Science* 271, 822-5.
7. Park, H. et al. (1996) *Immunity* 4, 515-25.
8. Kang, S.W. et al. (2001) *EMBO J* 20, 5692-702.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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