Revision 8

Store at -20C

#8547

Btk (D3H5) Rabbit mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, W-S, IP, IHC-Bond, IHC-P, FC-FP	H M	Endogenous	77	Rabbit IgG	#Q06187	695

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunoprecipitation	1:200
IHC Leica Bond	1:50 - 1:200
Immunohistochemistry (Paraffin)	1:50 - 1:200
Flow Cytometry (Fixed/Permeabilized)	1:100 - 1:400

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #94988.

Specificity / Sensitivity

Btk (D3H5) Rabbit mAb recognizes endogenous levels of total Btk protein.

Species predicted to react based on 100% sequence homology:

Rat, Hamster, Bovine, Dog, Pig, Horse

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp195 of human Btk protein.

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 nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation **IHC-Bond:** IHC Leica Bond **IHC-P:** Immunohistochemistry (Paraffin) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

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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Limited Uses