

Store at
-20°C

#79781

PhosphoPlus® Btk (Tyr223) Antibody Duet



Cell Signaling
TECHNOLOGY®

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Entrez-Gene ID #695
UniProt ID #Q06187

New 04/18

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
P-Btk (Y223) (D1D2Z) Rabbit mAb	87457	100 µl	78 kDa	Rabbit IgG
Btk (D3H5) Rabbit mAb	8547	100 µl	78 kDa	Rabbit IgG

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: PhosphoPlus® Duets from Cell Signaling Technology (CST) provide a means to assess protein activation status. Each Duet contains an activation-state and total protein antibody to your target of interest. These antibodies have been selected from CST's product offering based upon superior performance in specified applications.

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).

Specificity/Sensitivity: Btk (D3H5) Rabbit mAb recognizes endogenous levels of total Btk protein. Phospho-Btk (Tyr223) (D1D2Z) Rabbit mAb recognizes endogenous levels of Btk protein only when phosphorylated at Tyr223. The antibody detects a 26 kDa protein of unknown identity that is not sensitive to treatment with anti-IgM or Ibrutinib.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp195 of human Btk protein and a phosphopeptide corresponding to residues surrounding Tyr223 of human Btk protein.

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.

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.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey 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 All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.