

Phospho-Btk (Ser180) (3D3) Mouse mAb

✓ 100 µl
(10 western blots)

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rev. 08/25/10

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W Transfected	H, (M)	80 kDa	Mouse IgG1**

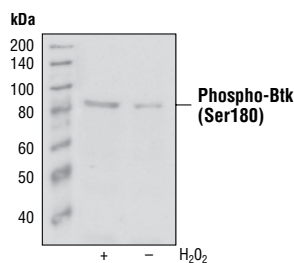
Background: Bruton's tyrosine kinase (Btk) is a member of the Btk/Tec family of cytoplasmic tyrosine kinases. Like other Btk family members, it contains a pleckstrin homology (PH) domain, and Src homology SH3 and SH2 domains. Btk plays an important role in B cell development (1,2). Activation of B cells 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-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 (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: Phospho-Btk (Ser180) (3D3) Mouse mAb detects transfected levels of Btk protein when phosphorylated at Ser180. For Western immunoblotting, CST recommends using immunoprecipitated Btk first.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser180 of human Btk.

Background References:

- (1) Khan, W.N. (2001) *Immunol. Res.* 23, 147–156.
- (2) Lewis, C.M. et al. (2001) *Curr. Opin. Immunol.* 13, 317–325.
- (3) Salim, K. et al. (1996) *EMBO J.* 15, 6241–6250.
- (4) Rameh, L.E. et al. (1997) *J. Biol. Chem.* 272, 22059–22066.
- (5) Varnai, P. et al. (1999) *J. Biol. Chem.* 274, 10983–10989.
- (6) Rawlings, D.J. et al. (1996) *Science* 271, 822–825.
- (7) Park, H. et al. (1996) *Immunity* 4, 515–525.
- (8) Kang, S.W. et al. (2001) *EMBO J.* 20, 5692–5702.



Western blot analysis of extracts from hydrogen peroxide treated or serum starved THP1 cells using Phospho-Btk (Ser180) (3D3) Mouse mAb.

Entrez-Gene ID # 695
Swiss-Prot Acc. # Q06187

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.

*Species cross-reactivity is determined by western blot.

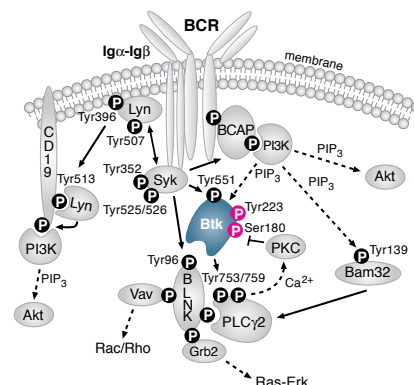
**Anti-mouse secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western Blotting 1:1000

For application specific protocols please see the web page for this product at www.cellsignaling.com.

Please visit www.cellsignaling.com for a complete listing of recommended companion products.



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

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: 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.