

#8547 Store at -20°C

Btk (D3H5) Rabbit mAb



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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, F Endogenous	H, M, (R, Hm, B, Dg, Pg, Hr)	77 kDa	Rabbit IgG**

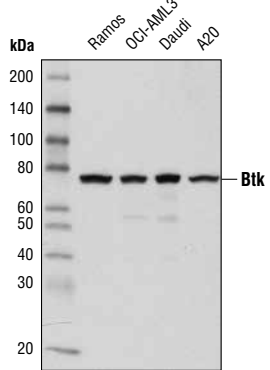
Background: Bruton's tyrosine kinase (Btk) is a member of the Btk/Tec family of cytoplasmic tyrosine kinases. Like other Btk family members, it contains a pleckstrin homology (PH) domain and Src homology SH3 and SH2 domains. Btk plays an important role in B cell development (1,2). Activation of B cells 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.

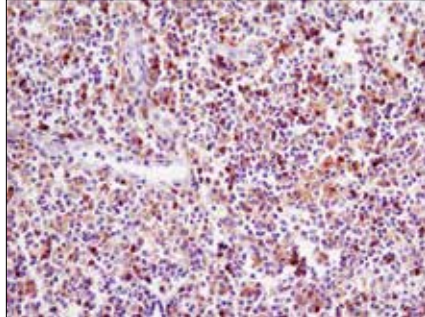
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp195 of human Btk protein.

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 various cell lines using Btk (D3H5) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human B-cell lymphoma using Btk (D3H5) Rabbit mAb.

Entrez-Gene ID #695
UniProt ID #Q06187

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.

*Species cross-reactivity is determined by western blot.
**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:200
Immunohistochemistry (Paraffin)	1:100†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunohistochemistry (Leica® Bond™)	1:100
Flow Cytometry	1:200
Flow Protocol:	Use Alternate Flow Protocol

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

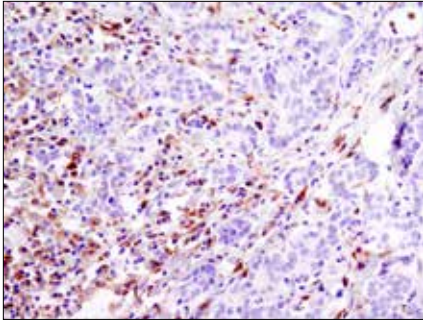
Please visit www.cellsignal.com for a complete listing of recommended companion products.

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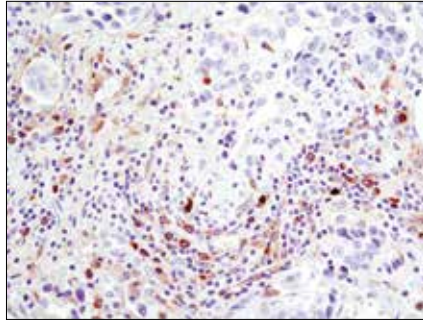
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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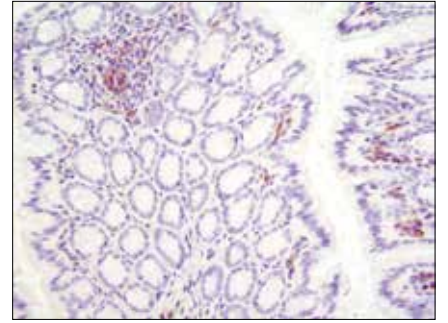
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA—Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
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.



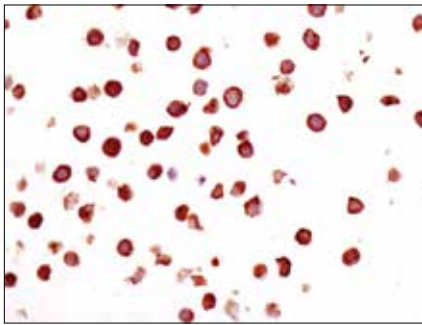
Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Btk (D3H5) Rabbit mAb. Note staining of inflammatory cells.



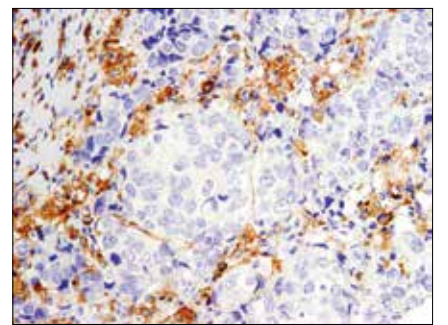
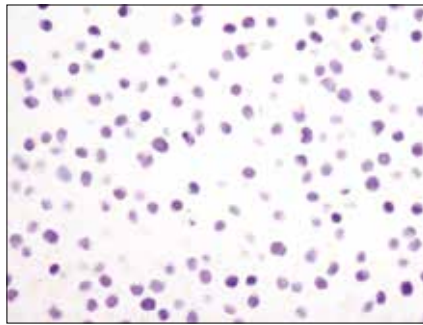
Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma using Btk (D3H5) Rabbit mAb. Note staining of inflammatory cells.



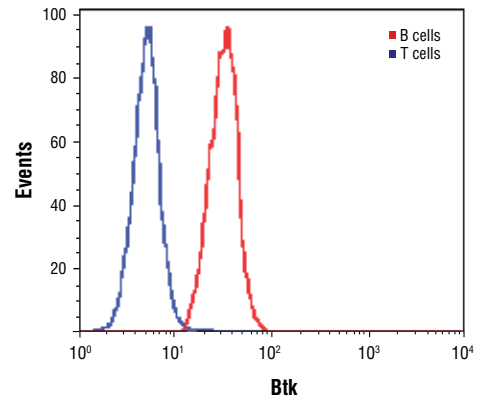
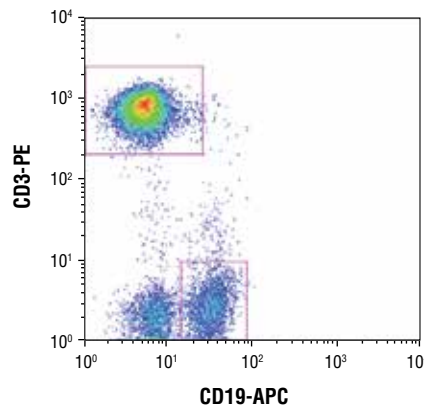
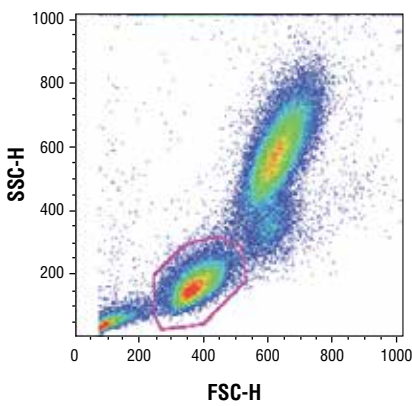
Immunohistochemical analysis of paraffin-embedded mouse colon using Btk (D3H5) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded cell pellets, Ramos (left) or Jurkat (right), using Btk (D3H5) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human squamous cell lung carcinoma using Btk (D3H5) Rabbit mAb performed on the Leica® Bond™ Rx.



Human whole blood was fixed, lysed, and permeabilized as per the Cell Signaling Technology Flow Alternate Protocol and stained using Btk (D3H5) Rabbit mAb. Samples were co-stained using CD3-PE and CD19-APC to distinguish T and B cell subpopulations, respectively. B (red) and T (blue) cell population gates were applied to a histogram depicting the mean fluorescence intensity of Btk. Anti-rabbit IgG (H+L), F(ab)₂ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.