Technical Data Sheet

PE Mouse Anti-Btk (pY551)/Itk (pY511)

Product Information

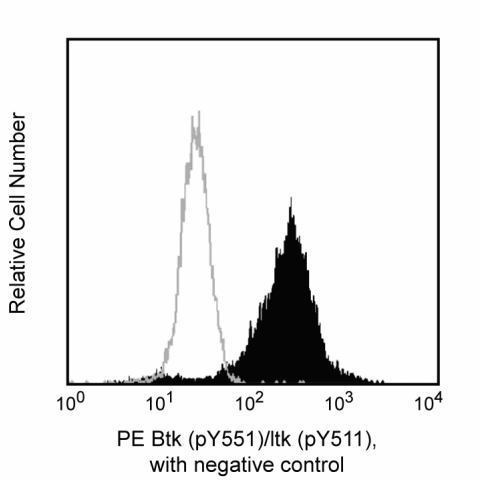
Material Number:	**558129**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	24a/BTK (Y551)
Immunogen:	Phosphorylated Human Btk Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Tested: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

*B*ruton's *t*yrosine *k*inase (Btk) is a nonreceptor tyrosine kinase whose function is critical for proper B cell development and signaling. The activity of Btk is regulated by Src mediated phosphorylation of the kinase domain at tyrosine 551 (Y551). This event induces Btk kinase activity and subsequent autophosphorylation at Y223. Phosphorylated Btk then associates with the cell membrane via the interaction of the PH domain with phosphatidylinositol 3, 4, 5-triphosphate.

The Tec family kinase Itk plays a critical role in signal transduction downstream of the T cell antigen receptor and has been implicated in the activation of phospholipase C-γ1 (PLCγ1), a key regulator of calcium mobilization and extracellular signal-regulated kinase (ERK) activation. Itk is regulated by an activating transphosphorylation event in which Y511 in the kinase domain is phosphorylated by Lck.

The 24a/BTK (Y551) monoclonal antibody recognizes the Y551-phosphorylated form of human Btk and the Y511 phosphorylated form of human Itk.



Analysis of Btk (pY551) in activated human B lymphoma cells. *Ramos cells (ATCC CRL-1596) were serum starved overnight and then either stimulated with 5 mM hydrogen peroxide for 15 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with PE anti-Btk (pY551)/Itk (pY511). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)

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Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. All other brands are trademarks of their respective owners.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Mahajan S, Fargnoli J, Burkhardt AL, Kut SA, Saouaf SJ, Bolen JB. Src family protein tyrosine kinases induce autoactivation of Bruton's tyrosine kinase. *Mol Cell Biol.* 1995; 15:5304-5311. (Biology)

Rawlings DJ, Scharenberg AM, Park H, et al. Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. *Science.* 1996; 271:822-825. (Biology)

Wilcox HM, Berg LJ.. Itk phosphorylation sites are required for functional activity in primary T cells. *J Biol Chem.* 2003; 278:37112-37121. (Biology)

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