Technical Data Sheet

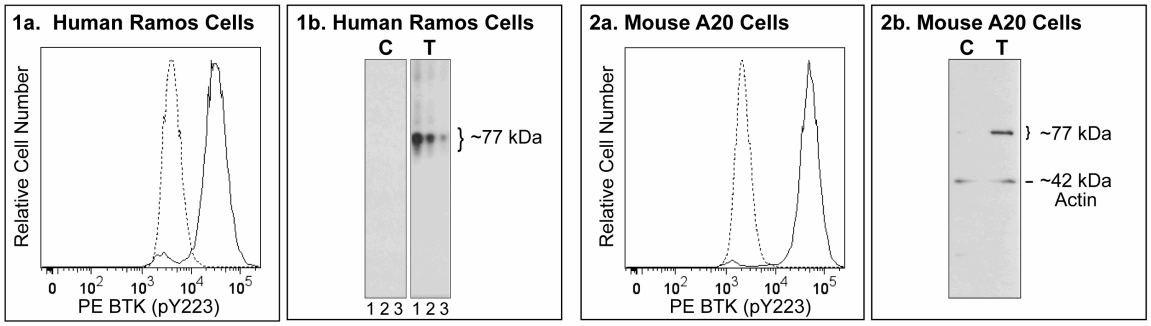
PE Mouse anti-Btk (pY223)/Itk (pY180)

Product Information

Material Number:	**562753**
Alternate Name:	Btk; Bruton tyrosine kinase; AGMX1; AT; ATK; BPK; IMD1; PSCTK1; XLA; Itk
Size:	50 tests
Vol. per Test:	5 µl
Clone:	N35-86
Immunogen:	Phosphorylated Human BTK Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse Predicted due to immunogen sequence identity: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The N35-86 reacts with human and mouse Bruton's tyrosine kinase that is phosphorylated at the tyrosine 223 position, Btk (pY223). Btk is also known as agammaglobulinaemia tyrosine kinase (ATK) and B-cell progenitor kinase (BPK). Btk is a nonreceptor tyrosine kinase whose function is critical for proper B cell development and signaling. It is a member of the Tec family of kinases which includes Tec and Itk. In addition to an N-terminal pleckstrin homology (PH) domain, the Tec proteins contain Src homology domains 2 and 3 (SH2 and SH3) and a stretch of 60-80 amino acids between the PH and SH3 domains termed the Tec homology domain. The activity of Btk is regulated by Src-mediated phosphorylation of the kinase domain at tyrosine 551. This event induces Btk kinase activity and subsequent autophosphorylation at tyrosine 223 in the SH3 domain. Phosphorylated Btk then associates with the cell membrane via the interaction of the PH domain with phosphatidylinositol 3, 4, 5-triphosphate. The PH domain is essential for proper activation and function of Btk. A mutation in the PH domain results in Xid, murine X-linked immunodeficiency, and human X-linked agammaglobulinemia. The orthologous phosphorylation site for rat BTK is Y224. Crossreactivity with human Itk (pY180) was confirmed by immunoprecipitation and Western blot analyses using the N35-86 antibody.



Analyses of BTK (pY223)/ ITK(pY180) expression by Human and Mouse Cells.

Human Cells

Panel 1a: *Flow cytometric analysis of BTK (pY223) expressed by human Ramos cells. Serum-starved Ramos cells (Human Burkitt's lymphoma cell line) were either not stimulated (dashed line histogram) or stimulated (solid line histogram) with Goat F(ab')2 Anti-Human IgM (Southern Biotech, Cat. No. 2022-14) at 37°C for 2 minutes. Cells were fixed in BD Cytofix™ Buffer (Cat. No. 554655; 37°C for 10 min) and permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice (30 min). Cells were then stained with BD Phosflow™ PE Mouse Anti-Btk (pY223)/Itk (pY180) (Cat. No. 562753). Histograms showing BTK (pY223) expression were generated for gated events with the forward and side-light scatter characteristics of intact cells using a BD FACSCanto™ II Flow Cytometer System.*

Panel 1b: *Western blot analysis of BTK (pY223) expressed by Ramos cells. Lysates from 1 million unstimulated (C) and Anti-IgM stimulated (T) Ramos cells were blotted using Purified Btk (pY223)/Itk (pY180) antibody (0.125, 0.063, and 0.032 µg/ml for Lanes 1, 2, and 3, respectively), HRP Goat Anti-Mouse Ig (Cat. No. 554002) and a chemiluminescent detection system. BTK (pY223) was identified as band of ~77 kDa.*

Mouse Cells

Panel 2a: *Flow cytometric analysis of BTK (pY223) expressed by mouse A20 cells. A20 cells (mouse B lymphoma cell line) were either not stimulated (dashed line histogram) or were stimulated (solid line histogram) with Rabbit Anti-Mouse IgG (H+L) (Jackson, Cat. No. 315-005-003) at 37˚C for 2 minutes. The cells were fixed, permed, stained and analyzed by flow cytometry as described above.*

Panel 2b: *Western blot analysis of BTK (pY223) expressed by mouse A20 cells. Lysates from 1 million untimulated (C) and Anti-IgM stimulated (T) A20 cells were blotted as described above with Purified Mouse Anti-Btk (pY223)/Itk (pY180) antibody at 0.032 µg/ml. Lower MW band at 42 kDa represents actin.*

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Preparation and Storage

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.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

The purified or fluorescent mAb was characterized by flow cytometry (Flow) and Western blot (Wb) using these model systems:

Method	Species	Cells	Treatment	Fixation	Perm bufferResult	Result
Flow	Human	Ramos (serum-starved)	Anti-human IgM	Cytofix or Lyse/Fix	Perm III	Induced, with higher S/N using Lyse/Fix than using Cytofix
	Human	Daudi (serum-starved)	Anti-human IgM	Cytofix or Lyse/Fix	Perm III	Induced, with higher S/N using Lyse/Fix than using Cytofix
	Human	PBMC	Anti-human IgM	Cytofix	Perm III	Induced only in CD20+ lymphocytes
	Mouse	A20	Anti-mouse IgG	Cytofix	Perm III	Induced
	Human	Ramos (serum-starved)	Anti-human IgM	Cytofix	Perm I, II, III, IV, 0.5x IV	Induced, working in all 5 Perm buffers
WB	Human	Ramos (serum-starved)	Anti-human IgM			Main band at 77 kDa induced
	Human	Ramos (serum-starved)	H_2O_2			Main band at 77 kDa induced
	Human	Jurkat (serum-starved)	H_2O_2			Main band at 77 kDa induced
	Human	Daudi (serum-starved)	Anti-human IgM			Main band at 77 kDa induced
	Human	PBMC	Anti-human IgM			Multiple bands with 77 kDa band induced in some donors
	Mouse	Mouse spleen cells	Anti-mouse IgM			77 kDa and 110 kDa bands induced
	Mouse	A20	Anti-mouse IgG			Main band at 77 kDa induced
IP-WB	Human	Jurkat (serum-starved)	H_2O_2			Crossreactivity with ITK (pY180)

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
560746	Perm Buffer IV 10×	50 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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Marshall AJ, Niito H, Yun TJ, Clark EA. Regulation of B-cell activation and differentiation by the phosphatidylinositol 3-kinase and phospholipase Cγ pathway. *Immunol Rev.* 2000; 176:30-46. (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)

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