Alexa Fluor® 488 Mouse Anti-Btk (pY223)/Itk (pY180)

Product Information

Material Number: 564847
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

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.

Left Panel - Flow cytometric analysis of BTK (pY223) expressed by human Ramos cells. Serum-starved cells from the human Ramos (Burkitt's lymphoma, ATCC CRL-1596) 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 3 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™ Alexa Fluor® 488 Mouse Anti-Btk (pY223)/Itk (pY180) antibody (Cat. No. 564847). Histograms showing BTK (pY223) expression were derived from gated events with the forward and side light-scatter characteristics of intact cells using a BD™ LSR II Flow Cytometer System.

Right Panel - Flow cytometric analysis of BTK (pY223) expressed by mouse A20 cells. Cells from the mouse A20 (B lymphoma, ATCC TIB-208) cell line were either not stimulated (dashed line histogram) or were stimulated (solid line histogram) with Rabbit Anti-Mouse IgM (H+L) (Jackson, Cat. No. 315-005-003) at 37°C for 3 minutes. The cells were fixed, permeabilized, stained, and analyzed for BTK (pY223) by flow cytometry as described above.

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**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 to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

**Application Notes**

**Application**

<table>
<thead>
<tr>
<th>Intracellular staining (flow cytometry)</th>
<th>Routinely Tested</th>
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**Suggested Companion Products**

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<td>Stain Buffer (FBS)</td>
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<td>557782</td>
<td>Alexa Fluor® 488 Mouse IgG1 κ Isotype Control</td>
<td>50 Tests</td>
<td>MOPC-21</td>
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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. An isotype control should be used at the same concentration as the antibody of interest.
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. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at wwwbdbiosciencescom/colors.
9. Please refer to wwwbdbiosciencescom/pharmingen/protocols for technical protocols.

**References**


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