

Technical Data Sheet

Alexa Fluor® 647 Mouse anti-BLNK (pY84)

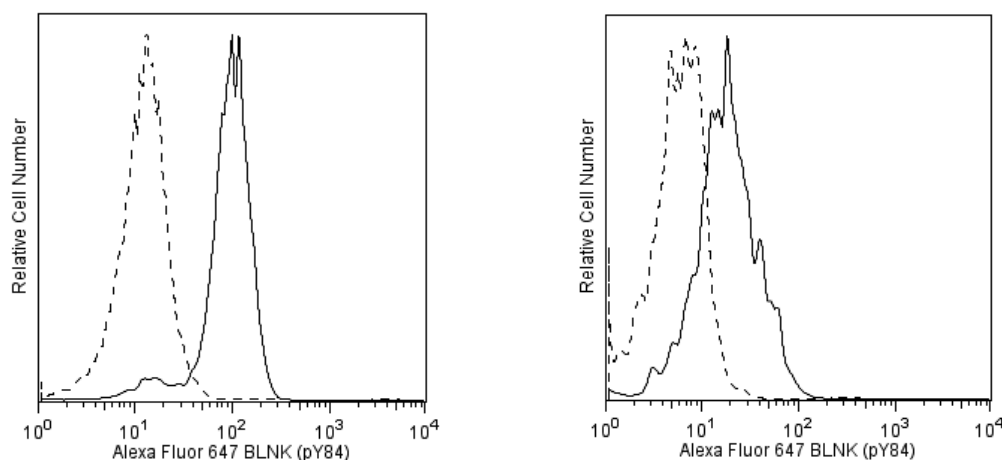
Product Information

Material Number:	558443
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J117-1278
Immunogen:	Phosphorylated Human BLNK Peptide
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

B cell activation is initiated by crosslinking the B cell receptor, which leads to activation of non-receptor protein tyrosine kinases (PTK), including Btk, Syk, and three Src kinases, Fyn, Lyn, and Blk. Activated PTKs then phosphorylate multiple cellular proteins involved in B lymphocyte signaling. Syk is responsible for the tyrosine phosphorylation of B cell linker protein (BLNK), a member of the SLP-76 family of adapter proteins. Phosphorylation of human BLNK at tyrosines 84, 178, and 189 (Y84, Y178, and Y189) creates docking sites for PLCγ2, leading to the activation of downstream signaling pathways.

The J117-1278 monoclonal antibody recognizes the phosphorylated Y84 of BLNK.



Analysis of BLNK (pY84) in human Burkitt's lymphoma and peripheral blood lymphocytes. Ramos cells (left panel) and human peripheral blood mononuclear cells (PBMC, right panel) were either stimulated (solid-line histograms) by cross-linking of surface IgM with purified Goat anti-Human IgM (SouthernBiotech) at 37°C for 2 minutes or unstimulated (dashed-line histograms). The cells were fixed with pre-warmed BD Cytotfix™ buffer (Cat. No. 554655) for 10 minutes at 37°C, then permeabilized with BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885) at room temperature for 30 minutes, and then stained with Alexa Fluor® 647 anti-BLNK (pY84) (both panels), APC anti-human CD3 mAb UCHT1 (Cat. No. 555335, bottom panel), and PerCP-Cy5.5 anti-human CD20 mAb H1(FB1) (Cat. No. 558021, bottom panel). For data analysis of PBMC, CD3-negative CD20-positive lymphocytes were selected by their staining and scatter profiles. 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 to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 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- μ l experimental sample (a test).
2. 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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
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® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

References

Chiu CW, Dalton M, Ishiai M, Kurosaki T, Chan AC. BLNK: molecular scaffolding through 'cis'-mediated organization of signaling proteins. *EMBO J.* 2002; 21:6461-6472. (Biology)

Espeli M, Mancini S, Breton C, Poirier F, Schiff C. Impaired B-cell development at the pre-BII-cell stage in galectin-1-deficient mice due to inefficient pre-BII/stromal cell interactions. *Blood.* 2009; 113(23):5878-5886. (Clone-specific: Flow cytometry)

Janssen E, Zhang W. Adaptor proteins in lymphocyte activation. *Curr Opin Immunol.* 2003; 15:269-276. (Biology)

Pighi C, Gu T, Dalai I, et al. Phospho-proteomic analysis of mantle cell lymphoma cells suggests a pro-survival role of B-cell receptor signaling. *Cell Oncol.* 2011; 34(2):141-153. (Clone-specific: Flow cytometry, Western blot)

Wu JN, Koretzky GA. The SLP-76 family of adapter proteins. *Semin Immunol.* 2004; 16:379-393. (Biology)

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