Technical Data Sheet

Alexa Fluor® 647 Mouse anti-BLNK

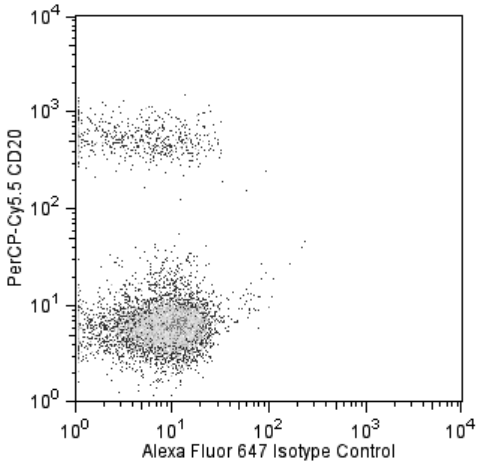
Product Information

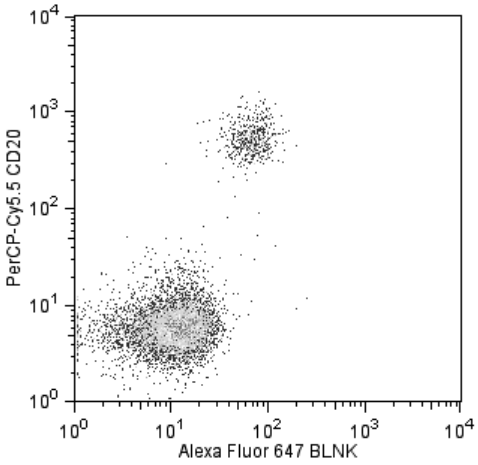
Material Number:	**558692**
Alternate Name:	SLP-65, BASH, BCA
Size:	50 tests
Vol. per Test:	20 µl
Clone:	2B11
Immunogen:	Human N-terminal BLNK Recombinant Protein
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Tested: Human
	Confirmed during development: Mouse
Storage Buffer:	Aqueous buffered solution containing 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 *lin*ker 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 2B11 monoclonal antibody recognizes BLNK, regardless of phosphorylation status. A fusion protein representing amino acids 4-205 of human BLNK was used as the immunogen. BLNK is expressed as two phosphoproteins migrating at 68 and 70 kDa in SDS/PAGE that represent alternatively spliced forms of human BLNK.





Analysis of BLNK in human peripheral blood lymphocytes. *Human whole blood was lysed and fixed with 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 10-15 minutes at 37°C, and the leukocytes were permeabilized with BD Phosflow™ Perm Buffer II (Cat. No. 558052) on ice for 30 minutes. The cells were then stained with either Alexa Fluor® 647 Mouse IgG2a, κ, isotype control (left panel) or Alexa Fluor® 647 Mouse anti-BLNK (right panel). B lymphocytes were identified by their scatter profile and staining with PerCP-Cy5.5 Mouse anti-human CD20 (cytoplasmic) (Cat. No. 558021). BLNK expression was restricted to the CD20-positive B cells. 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

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human whole blood (using BD Phosflow™ Lyse/Fix Buffer) and peripheral blood mononuclear cells (using BD Cytofix™ Fixation Buffer). Any of the three BD Phosflow™ permeabilization buffers may be used.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558052	Perm Buffer II	125 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21
558021	PerCP-Cy™5.5 Mouse Anti-Human CD20	50 tests	H1

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. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
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.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. An isotype control should be used at the same concentration as the antibody of interest.
8. 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.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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

Li X, Martin F, Oliver AM, Kearney JF, Carter RH. Antigen receptor proximal signaling in splenic B-2 cell subsets. *J Immunol.* 2001; 166:3122-3129. (Clone-specific)

Minegishi Y, Rohrer J, Coustan-Smith E, et al. An essential role for BLNK in human B cell development. *Science.* 1999; 286:1954-1957. (Immunogen: Flow cytometry)

Taguchi T, Kiyokawa N, Takenouch H, et al. Deficiency of BLNK hampers PLC-γ2 phosphorylation and Ca2+ influx induced by the pre-B-cell receptor in human pre-B cells. *Immunology.* 2004; 122:575-582. (Clone-specific: Flow cytometry)

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

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