Technical Data Sheet

Alexa Fluor® 488 Mouse anti-CD22 (pY822)

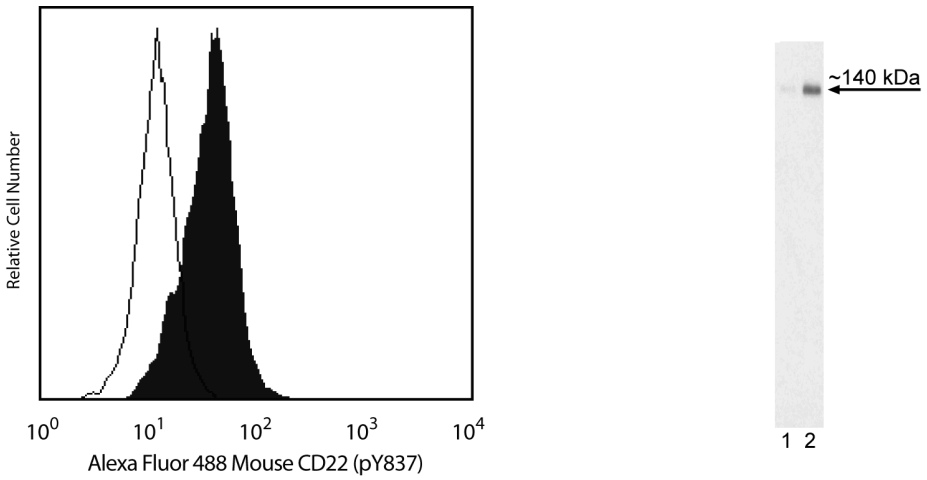
Product Information

Material Number:	**560075**
Alternate Name:	Human BL-CAM, Siglec-2, or Leu-14 (pY822); Mouse Lyb-8 (pY837)
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	12a/CD22
Immunogen:	Phosphorylated Mouse CD22 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

CD22 is a member of the immunoglobulin (Ig) superfamily that contains several extracellular Ig domains and cytoplasmic *I*mmunoreceptor *T*yrosine-based *I*nhibitory *M*otifs (ITIMs). It is expressed in the cytoplasm of pro-B and pre-B cells and on the surface of IgD-positive mature B lymphocytes. Differential splicing of the human CD22 gene results in the expression of two isoforms; the full-length CD22β has 7 Ig domains, while CD22α has only 5. CD22β is the predominant isoform in human cells, at both the mRNA and protein levels. Ligands for the extracellular domains of CD22 are sialic acid-linked glycoproteins on the B cell surface. The intracellular ITIM motifs are substrates of src family tyrosine kinases. Signal transduction is initiated by the phosphorylation of tyrosines in the ITIMs of CD22 upon ligation of the B cell receptor (BCR). Tyrosine phosphorylation in ITIM3 of human CD22 or ITIM2 of mouse CD22 is required for recruitment of the protein tyrosine phosphatase PTP1C (SHP-1), which may terminate signal transduction by CD22 and by co-localized receptors, such as the BCR. Studies of mice deficient for CD22 suggest that this adhesion molecule generates both negative and positive regulatory signals that affect B cell development and responsiveness.

The 12a/CD22 monoclonal antibody recognizes the phosphorylated tyrosine 822 (pY822) in ITIM3 of human CD22β. The equivalent site in human CD22α is pY645. The orthologous phosphorylation site in the predominant isoform of mouse CD22 is pY837, which is in ITIM2. We recommend the PE conjugate of mAb 12a/CD22, Cat. No. 560076, for staining human peripheral blood mononuclear cells (PBMC).



Analysis of CD22 (pY837) in mouse B lymphocytes.
LEFT: *Mouse splenocytes were suspended in 1× PBS containing 10% fetal calf serum and either stimulated by cross-linking of surface IgM with purified F(ab')2 Goat Anti-Mouse IgM (Jackson Immunoresearch, shaded histogram) at 37°C for 2 minutes or unstimulated (open histogram). The cells were fixed with BD Phosflow™ Lyse/Fix buffer (Cat. No. 558049) for 10-15 minutes at 37ºC, then permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 488 Mouse anti-CD22 (Cat. No. 560075) and Alexa Fluor® 647 Rat Anti-Mouse CD45R/B220 (Cat. No. 557683). For data analysis, CD45R/B220-positive B lymphocytes were selected by their staining and scatter profiles (data not shown). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*
RIGHT: *The specificity of mAb 12a/CD22 was confirmed by western blot analysis using unconjugated antibody at 0.5 µg/ml on lysates from control (lane 1) and anti-IgM-stimulated (lane 2) mouse splenocytes. CD22 (pY837) (mouse) is identified as a band of ~140 kDa in the activated cells.*

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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

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 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. 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.
4. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Fujimoto M, Kuwano Y, Watanabe R, et al. B cell antigen receptor and CD40 differentially regulate CD22 tyrosine phosphorylation. *J Immunol.* 2006; 176:873-879. (Biology: Depletion, Flow cytometry)

Grewal :K, Boton M, Ramirez K, et al. ST6Gal-I restrains CD22-dependent antigen receptor endocytosis and Shp-1 recruitment in normal and pathogenic immune signaling. *Mol Cell Biol.* 2006; 26(13):4970-4981. (Biology)

Tedder TF, Tuscano J, Sato S, Kehrl JH. CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling. *Annu Rev Immunol.* 1997; 15:481-504. (Biology)

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