OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM39016PU-N

CD22 Mouse Monoclonal Antibody [Clone ID: B-ly8]

Product data:

Product Type:	Primary Antibodies
Clone Name:	B-ly8
Applications:	FC, IF, IHC
Recommended Dilution:	CD22 antibodies are used as a pan B cell reagent, for the immunophenotyping of B cell lymphomas and HCL. Anti-CD22, clone B-ly8, can be applied in flow cytometry for analysis of blood and bone marrow samples, or in immunohistochemistry using cytospots or frozen tissue sections. Flow cytometry: please see "Protocols" below. Labelled reagent is effectively formulated for direct immunofluorescent staining.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Specificity:	Clone B-ly8 produces mouse IgG1 immunoglobulins directed against CD22, molecular weight 130-140 kD.
Formulation:	0.01M Sodium Phosphate, 0.15M NaCl, pH 7.3, 0.2% BSA, 0.09% Sodium Azide State: Aff - Purified State: Liquid purified Ig fraction Label: Cat. No. Label EX-max (nm) / EM-max (nm): AM39016FC-N FITC 488 / 519 AM39016RP-N 488, 532 / 578 AM39016PU-N Pure . /
Concentration:	lot specific
Purification:	Affinity Chromatography
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C. Fluorochrome labelled product is photosensitive and should be protected from light.
Stability:	Shelf life: one year from despatch.
Gene Name:	CD22 molecule

Database Link: Entrez Gene 933 Human
P20273

Background: CD22 is detected in the cytoplasm early in B cell development (late pro-B cell stage), appears on the cell surface simultaneously with surface IgD, and is found on most mature B cells. Expression is lost with terminal differentiation of B cells and is absent on plasma cells. Activation of B cells via surface Ig increases CD22 expression [1]. It is more strongly expressed on prolymphocytic leukemia and HCL than in chronic lymphocytic leukemia. B cell lineage ALL express membrane and cytoplasmic CD22.
CD22 forms a loose complex with the BcR cell antigen receptor (BcR) [2]. The cytoplasmic domain is tyrosine phosphorylated upon ligation of the BCR and associates via SH2 domains with the tyrosine phosphatase SHP-1, the tyrosine kinase Syk and phospholipase C-g1 [3,4]. CD22 down-modulates the B cell activation threshold, presumably through its association with SHP-1 and other signaling molecules [2,3]. Mice deficient in CD22 show exaggerated antibody responses to antigen and have raised levels of autoantibodies [1,5]. CD22 can also mediate cell adhesion through its interaction with cell surface molecules bearing the appropriate sialoglycoconjugates, but only when these conjugates are not on the CD22 bearing cell itself [2,6].

Synonyms: SIGLEC2, Siglec-2, B-cell receptor CD22, Leu-14, BL-CAM

Note: 1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion.
3. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Protocol: Flow cytometry method for use with labelled (FITC, R-PE, APC, PerCP or PerCP-Cy5.5) monoclonal antibodies
1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of labelled monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study).
Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Add 100 µl of a lyse reagent.
5. Incubate for 10 minutes at room temperature in the dark.
6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
8. Remove the supernatant and resuspend the cells in 200 µl of PBS.
9. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed

upon fixation and this should be taken into account when using this alternative).

Flow cytometry method for use with dual and triple combinations

1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
For combinations with anti-kappa and/or anti-lambda Ig see **Application note** below.
2. Add to each tube 20 µl of labelled monoclonal antibody combination.
(Appropriate mouse Ig isotype control samples should always be included in any labelling study).
3. Vortex the tube to ensure thorough mixing of antibody and cells.
4. Incubate the tube for 15 minutes at room temperature in the dark.
5. Add 100 µl of a lyse reagent and mix immediately.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
8. Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.
9. Remove the supernatant and resuspend the cells in 200 µl of PBS.
10. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Application note for anti-kappa and/or anti-lambda Ig combinations

Add 2 ml of PBS containing 0.001% (v/v) Heparin (prewarmed to 37°C) to the cell suspension
Vortex, centrifuge (2 min at 300x g) and discard the supernatant.
Repeat this step twice.
Resuspend the pelleted blood cells in 100 µl PBS, pH 7.2, containing 0.001% (v/v) Heparin.

Flow cytometry method for use with purified monoclonal antibodies

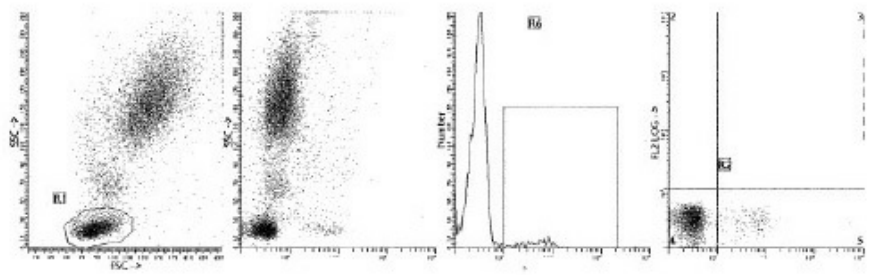
1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of purified monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study).
Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Wash the labelled cells by adding 2 ml of PBS containing 0.001% (v/v) Heparin, vortexing and centrifuging (2 min 1000 x g) and discard the supernatant.
5. Add 50 µl of appropriate dilution of F(ab)2 Rabbit Anti Mouse IgG fluorescent conjugate (e.g.FITC or R-PE) in PBS containing 0.001% (v/v) Heparin to the tube. It is recommended that the tube is protected from light.
6. Mix by vortexing and incubate for 15 minutes at room temperature in the dark.
7. Add 100 µl of a lyse reagent and mix immediately.
8. Incubate for 10 minutes at room temperature in the dark.
9. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
10. Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.
11. Remove the supernatant and resuspend the cells in 200 µl of PBS.
12. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05%

of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Protein Families: Druggable Genome, Transmembrane

Protein Pathways: B cell receptor signaling pathway, Cell adhesion molecules (CAMs), Hematopoietic cell lineage

Product images:



Representative Data Clone B-ly8 (CD22) was analyzed by flow cytometry using a blood sample from a healthy volunteer. Indirect staining was performed by adding 10 µl unlabelled monoclonal antibody to 100 µl blood sample, followed by FITC-labelled secondary antibody.