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Product datasheet for AM39015PU-N

CD21 (CR2) Mouse Monoclonal Antibody [Clone ID: B-ly4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	B-ly4
Applications:	FC, IHC
Recommended Dilution:	Anti-CD21, clone B-ly4, can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry on frozen or paraffin embedded 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-ly4 produces mouse IgG1 immunoglobulins directed against a 145 kD polypeptide of the C3d receptor. Clone B-ly4 was clustered at the Leucocyte Typing Workshop IV [5].
Formulation:	0.01 M sodium phosphate, 0.15 M 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): AM39015FC-N FITC 488 / 519 AM39015RP-N 488, 532 / 578 AM39015PU-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:	complement component 3d receptor 2

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Database Link: Entrez Gene 1380 Human
P20023

Background: The CD21 antigen is expressed on mature B cells, follicular dendritic cells and pharyngeal and cervical epithelial cells [1,2]. CD21, also known as the Complement receptor type 2 (CR2) or Epstein-Barr virus (EBV) receptor, is a receptor for the C3 activation fragments iC3b and C3d. CD21 is part of a multimeric complex on B cells which includes CD19 and CD81 [3]. The ligand for CD21 is generated during activation of complement by the attachment of C3d to potential antigen. This creates fusion proteins that may cross-link the CD19-CD21-CD81 complex to sIg on antigen-specific B cells. The resulting signal transduction lowers the threshold for cellular activation. This enables an antibody reaction to natural protein antigens containing only one or a few epitopes and therefore unable to effectively cross-link Ig molecules on specific B cells. For example, keyhole limpet hemocyanin (KLH) immune complexes can be taken up and processed by all CR2-bearing B cells, regardless of their antigen specificity. KLH immune complexes taken up this way by non-specific B cells were shown to be presented to KLH-specific, MHC class II Th cells. CD23 may also serve as a ligand for CD21 [3].

Synonyms: Complement receptor type 2, CR2, C3DR, C3d receptor, EBV Receptor, Dendritic Cell Marker

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

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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).

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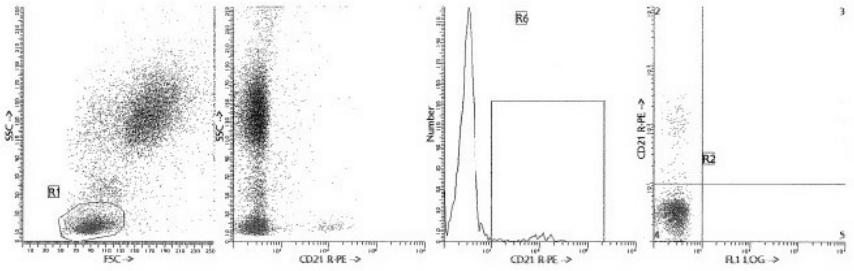
Protein Families: Druggable Genome, Transmembrane

Protein Pathways: B cell receptor signaling pathway, Complement and coagulation cascades, Hematopoietic cell lineage

Product images:



Representative Data Clone B-ly4 (CD21) was analyzed by flow cytometry using a blood sample obtained from a healthy volunteer. Direct staining was performed using 10 µl of the PE-conjugated monoclonal antibody and 100 µl of blood sample.

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