CD21 antibodies

Analyte specific reagents (ASR)

Analytical and performance characteristics were not established

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1. General information

Intended use
HB5 reacts with human CD21. The fluorescently labeled CD21 antigen can be detected by flow cytometry.

Reagents and contents
Monoclonal CD21 antibody conjugates

Product	Volume	REF
CD21-VioBlue	0.5 mL	170-081-022
CD21-PE	0.5 mL	170-081-037

2. Technical data and background information

Antigen	CD21
Clone	HB5
Isotype	Murine IgG2a, κ light chain
Alternative names of antigen	Complement Receptor 2 (CR2), Complement 3d Receptor
Purification	Affinity chromatography
Product formulation	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
+2°C +8°C	Store at +2 °C to +8 °C (+36 °F to +46 °F).
	Store protected from light.
	The use-by date is indicated on the vial label.
	For in-use stability at +2 °C to +8 °C (+36 °F to +46 °F) storage temperature refer to the use-by date indicated on the vial label. Do not use the reagent after the use-by date.

Expression pattern
HB5 recognizes CD21, a type I membrane glycoprotein. Expression of CD21 in humans is found on B cells, follicular dendritic cells, subsets of epithelial cells, and thymic T cells. The primary function attributed to CD21 has been to amplify the B cell receptor (BCR)-mediated signal transduction in response to antigen recognition. To enhance the BCR mediated activation, CD21 associates with CD19 and CD81 in a B cell–specific signal transduction complex, where interaction of BCR with CR2/CD19 amplifies the signals. In addition, CD21 serves as a receptor for split products of complement protein C3, the gp350/220 viral coat protein of the EBV and the immunoregulatory protein CD23.

3. Warnings and precautions

- Interpretation of results is under the full responsibility of the user.
- For all handling, consideration of good laboratory practice (GLP) regulations is recommended.
- Use of the reagents is restricted to trained and qualified personnel only.
- All biological specimens and all materials that come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment and disposal of infectious material must be followed.
- Reagents contain sodium azide (NaN_3), a chemical highly toxic in pure form. However, at product concentrations, it is not classified as hazardous. Sodium azide may react with lead and copper plumbing to form highly explosive buildups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing. Safety guidelines must be observed.
- For material required but not provided the manufacturers recommendations and safety regulations must be followed.
- Reagents should not be used if signs of leakage are observed. Use undamaged and sealed vials only.

4. Application

Reagents can be used for immunophenotyping by flow cytometry and other research applications.

5. General Use considerations

Principle of method:
The antibody reagent provided enables the identification of a specific target cell type by flow cytometry. This technique is based on fluorochrome conjugated antibodies binding to specific antigens expressed by the target cells. Incubating a sample of interest, e.g., peripheral blood mononuclear cells (PBMC), with the provided antibody reagent leads to fluorescent staining of the cell type expressing the specific target antigen. Analysis of the sample is performed in a flow cytometer at a single-cell level. The analysis is based on the detection of characteristic light emission patterns emitted by the fluorescently labeled antibody upon excitation with laser light. The collected data can be processed and analyzed using flow cytometry software.

Important notes:
Exposure of reagents to temperatures below +2 °C (+36 °F) and above +8 °C (+46 °F) and to light should be minimized during handling.

Sample requirements
- Reagents can be used for determination of antigen-positive cells in whole blood samples by flow cytometry.
- Each cell source can have different storage conditions and limitations that should be considered prior to collection and analysis. For collection of patient samples national legislation must be followed.
- Whole blood samples should be stained within 24 hours.
- Viability of the cells should be assessed and use of samples with at least 80% viable cells is suggested in order to minimize risk of erroneous results.

Quality control:
It is recommended to run regularly a control sample from a normal adult specimen or commercially available whole blood control as a quality control of the system.

6. Analytical specificity

HB5 was tested in the HLDA workshop 2 and proven to specifically recognize human CD21. The results have been published and referenced in Reinherz, E.L. *et. al.*, (eds) 1986, Leukocyte Typing II, New York, Springer-Verlag New York and Schlossman, S.F. *et. al.* (eds) 1995, Leukocyte Typing V, Boston, Oxford University Press.

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7. Excitation and emission data of fluorochrome conjugates

Fluorochrome	Excitation laser (nm)	Excitation maximum (nm)	Emission maximum (nm)
VioBlue®	405	400	452
VioGreen™	405	388	520
VioBright™ FITC	488	496	522
FITC	488	495	520
PE	488 or 561	565	578
PE-Vio®615	488 or 561	565	619
PerCP	488	482	675
PerCP-Vio®700	488	482	676
PE-Vio®770	488 or 561	565	775
APC	561 or 635	652	660
APC-Vio®770	561 or 635	652	775

8. Limitations

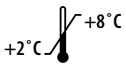
Use of monoclonal antibodies in patient treatment can interfere with recognition of target antigens by this reagent. This should be considered when analyzing samples from patients treated in this fashion. Miltenyi Biotec has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.

Reagent data was collected typically with EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

9. References

1. Barrington, R. A. *et al.* (2005) CD21/CD19 coreceptor signaling promotes B Cell survival during primary immune responses. J. Immunol. 175: 2589-2867.
2. Cherukuri, A. *et al.* (2001) The role of the CD19/CD21 complex in B cell processing and presentation of complement-tagged antigens. J. Immunol. 167: 163-172.
3. Braun, M. *et al.* (1998) Human B and T lymphocytes have similar amounts of CD21 mRNA, but differ in surface expression of the CD21 glycoprotein. Int. Immunol. 10: 1197-1202.
4. Roberts, M. L. *et al.* (1996) Epstein—Barr virus binding to CD21, the virus receptor, activates resting B cells via an intracellular pathway that is linked to B cell infection. Virology 77: 3077-3085.
5. TedderTF, Clement LT, Cooper MD. Expression of C3d receptors during human B cell differentiation: Immunofluorescence analysis with the HB-5 monoclonal antibody. J. Immunol. 1984; 133:678-683. Reinherz, E.L. *et al.*, (eds.) 1985, Leukocyte Typing II New York, Springer.

10. Glossary of symbols

	Manufacturer		Use-by date
	Order number		In-use stability
	Batch code		Temperature limit
	Consult instruction for use		Protect from sunlight
	Phone		Website

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