

#### PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD22 FITC CD5 R-PE RUO REF IOP-242FR 50 tests

RUO For Research Use Only

## **Description**

CD22 antibodies are used in flow cytometry and immunohistochemistry as a pan B cell reagent, for the immunophenotyping of B cell lymphomas and HCL. It is more strongly expressed on prolymphocytic leukemia and HCL than in chronic lymphocytic leukemia. B cell lineage ALL, express membrane and cytoplasmic CD22.

CD5 is used in the identification and localization of T cells in tissue and the diagnosis of T cell lymphomas and of B cell lymphocytic lymphomas of CLL types. In flow cytometry it may be used for the enumeration of T cells and CD5 positive B cells in peripheral blood. CD5 may also be applied in the depletion of T cells or CD5+ cells by complement mediated cytotoxicity.

**CD22** 

Clone B-ly8

**Isotype** Murine IgG1

Specificity

CD22 forms a loose complex with the BcR cell antigen receptor (BcR). 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. CD22 down-modulates the B cell activation threshold, presumably through its association with SHP-1 and other signaling molecules. Mice deficient in CD22 show exaggerated antibody responses to antigen and have raised levels of autoantibodies. CD22 can also mediate cell adhesion through its interaction with cell surface molecules bearing the appropriate sialoglyco-conjugates, but only when these conjugates are not on the CD22 bearing cell itself.

## **HLDA Workshop**

The Leukocyte Antigen Factsbook - Barclay, A.J. et.al., Academic Press. London (1997)

CD5

Clone MCD5

**Isotype** Murine IgG2b

**Specificity** CD5 is

CD5 is present on all mature T cells and 95% of thymocytes. Anti-CD5 also reacts with a distinct subset of normal B cells and most CLL cells, but with few other B cell leukemias and lymphomas. CD5 appears to be a relatively late marker during B cell differentiation. CD5 expression is thought to be absent on surface Ig negative B-lineage cells but appears on IgM+ cells in both

fetal liver and bone marrow.

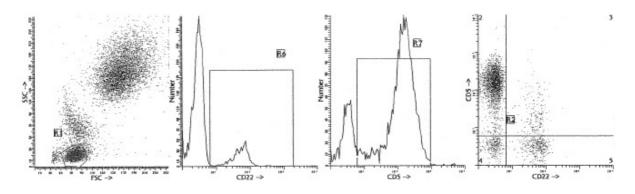
Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using  $10 \, \mu l/10^6$  leukocytes for singles and  $20 \, \mu l/10^6$  leukocytes in case

of dual and triple combinations. Since applications vary, each investigator should titrate the

reagent to obtain optimal results.

### **Representative Data**

IQP-242FR (CD22/CD5) was analyzed by flow cytometry using a blood sample from a healthy volunteer. Direct staining was performed by adding 20  $\mu$ l of this dual to 100  $\mu$ l blood sample.



### Immunofluorescence staining and lysing protocol

#### Flow cytometry method for use with dual and triple combinations

- 1. Add 100  $\mu$ l of EDTA-treated blood (i.e. approx.  $10^6$  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 labeled monoclonal antibody combination\*.
- 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 IQ Lyse (IQP-199 ready-to-use) 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 labeled 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).
  - \* Appropriate mouse Ig isotype control samples can be included in any labeling study \*\* PBS: Phosphate Buffered Saline, pH 7.2

#### 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 containing 0.001% (v/v) Heparin

# **△ ♦ / \* 2**

# **Handling and Storage**

Antibodies are supplied either as 100 tests per vial (1 ml) for singles or 50 tests per vial (1 ml) for dual and triple combinations. They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% BSA, 0.09% sodiumazide (NaN $_3$ ). Store the vials at 2-8 °C. Monoclonal antibodies should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly.

#### Warranty

Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product.

# Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

### Warning

All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only.

**Explanation of used symbols** 

(li Consult instructions for use REF Catalogue number Sufficient for

IVD In Vitro Diagnostic medical device  $\overline{\mathbb{A}}$ Caution, consult accompanying document

\* Keep away from (sun)light

8 Biological risks

∦ RUO Temperature limitation (°C) For Research Use Only LOT Batch code

Use by yyyy-mm-dd Manufacturer

EC REP Authorized Representative in the European Community

Conformité Européenne (European Conformity)

		Label - tandem	Ex -max (nm)	Em -max (nm)
P	PURE	purified material	-	-
F	FITC	FITC	488	519
R	R-PE	PE	488, 532	578
С	CyQ	PE-Cy5.18	488, 532	667
Α	APC		595, 633, 635, 647	660
PC	PerCP		488, 532	678
PCC	PerCP-Cy5.5		488, 532	695

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