



## Product Information

### MONOCLONAL ANTI-HUMAN CD22

#### Clone HIB22

Purified Mouse Immunoglobulin

Product Number **C 8464**

#### Product Description

Monoclonal Anti-Human CD22 (mouse IgG1 isotype) is derived from the HIB22 (HI22) hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Hairy Cell Leukemia cells were used as the immunogen. The isotype is determined using ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Human CD22 recognizes the human CD22 antigen expressed on the surface of mature B cells and in the cytoplasm of virtually all B cells, but not plasma cells. It is strongly expressed in Hairy Cell Leukemia cells.

The human CD22 antigen (BL-CAM) is a 135 kDa transmembrane phosphoglycoprotein that belongs to the immunoglobulin (Ig) superfamily. It may occur in two different isoforms: CD22 $\alpha$  chain with five Ig-like extracellular domains and CD22 $\beta$  chain with seven Ig-like extracellular domains. CD22 expression is restricted to normal and neoplastic B cells. It is expressed at the late pro-B stage shortly after CD19.<sup>1,2,3</sup> CD22 is largely found in early B cell development as a cytoplasmic protein. In mature B cells it is found on the cell surface. Plasma cells do not express this antigen. CD22 is strongly expressed on Hairy Cell Leukemia cells. It is also expressed in the cytoplasm and cell surface of most acute lymphoblastic leukemia precursor B cells. The antigen is demonstrable in primary and secondary follicles and in scattered extrafollicular B cells in frozen sections of human lymphoid tissue. CD22 is an adhesion molecule that mediates adhesion to erythrocytes, B cells, T cells, monocytes, and neutrophils. It is a lectin that specifically recognizes oligosaccharides with  $\alpha$ -2,6-linked sialic acids. CD75 antigen on B cells and CD45RO antigen on T cells are ligands for CD22. CD22 is involved in signal transduction in B cells activated through surface Ig. Monoclonal Anti-Human CD22 binds to an epitope dependent on the presence of both the first and the second Ig-like domains of the

CD22 molecule.<sup>4,5</sup> The antibody can be used for detection of both surface membrane and cytoplasmic CD22. It does not inhibit CD22 mediated adhesion of various cells to CD22 transfected cells.<sup>5</sup>

#### Reagents

The product is provided as Protein A purified antibody in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

#### Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

#### Storage/Stability

Store at 2 to 8 °C. Do Not Freeze. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

#### Procedure

##### Indirect Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied

1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A, or heparin anticoagulant OR  
b. Human cell suspension [peripheral blood mononuclear cells isolated on HISTOPAQUE® (Product Code 1077-1)].
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN<sub>3</sub>.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Product No. F 2883, FITC-Sheep Anti-Mouse IgG (whole molecule), F(ab')<sub>2</sub> fragment of Affinity Isolated Antibody). Aggregates in conjugates should be removed by centrifugation immediately prior to use.

4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. M 5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipette.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Product No. T 0776) in 0.01 M phosphate buffered saline, pH 7.4.
10. 2% paraformaldehyde in PBS.
11. Whole blood lysing solution.
12. Flow cytometer.

#### Procedure

1.
  - a. Use 100 µl of whole blood **or**
  - b. Adjust cell suspension to  $1 \times 10^7$  cells/ml in Diluent. Cells should be >90% viable as determined by dye exclusion (trypan blue). For each sample, add 100 µl or  $1 \times 10^6$  cells per tube.
2. Add 5 µl of monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 to 22 °C) for 30 minutes. Proper controls to be included for each sample are:
  - a. Autofluorescence control: 5 µl of Diluent in place of monoclonal antibody. Negative staining control : 5 µl of isotype-matched non-specific mouse immunoglobulin (Product No. M 5284) at the same concentration as test antibody.
3. After 30 minutes, add 2 ml of Diluent to all tubes.
4. Pellet cells by centrifugation at 500 x g for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml of Diluent.
7. Repeat washing procedure (steps 4-6).
8. After the second wash, resuspend the cells in 100 µl of the fluorochrome conjugated secondary antibody at the recommended concentration. For the autofluorescence control, add 100 µl of Diluent. Incubate at room temperature (18 to 22 °C) for 30 minutes. Protect from light at this and all subsequent steps.
9.
  - a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 10.
  - b. If a mononuclear cell suspension is used, proceed to Step 10.
- c. Add 2 ml of Diluent to all tubes.
10. Wash as in steps 4-6 twice.
11. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and analyze in a flow cytometer according to manufacturer's instructions.

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems it may be necessary to incubate the cells in 10 to 20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

#### **Product Profile**

When assayed by flow cytometric analysis (with a FACScan flow cytometer) using 5 µl of the antibody to stain  $1 \times 10^6$  cells, or 100 µl whole blood, a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels. The percent population positive is also at the maximum percentage positive using saturating monoclonal antibody levels.

Monoclonal Anti-Human CD22 may be used for:

1. Detection and enumeration of B cells in blood and tissues in health and disease.
2. Studies of cell adhesion and activation.
3. Typing of mature leukemias.

## References

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