

PN IM1871**100 tests
20 µL/test****CD34 - PE****(581)****IOTest[®]**
Conjugated Antibodies

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY

The molecular weight of the recognized antigen is about 110 kDa. The CD34 glycoprotein is a transmembrane single chain molecule (for reviews, see ref. 1,2,3). The extracellular domain is heavily N- and O-glycosylated (4,5,6,7) and the cytoplasmic sequence reveals two sites for activated protein kinase C phosphorylation and one site for tyrosine phosphorylation (6). This antigen is the earliest known marker in human for hematopoietic progenitor cells (8,9).

There are three classes of CD34 epitopes defined by differential sensitivity to enzymatic cleavage with glycoprotease from *Pasteurella haemolytica* and with neuraminidase (7).

The 581 monoclonal antibody recognizes specifically a class III epitope, neuraminidase- and glycoprotease-resistant (10,11).

Tissue distribution

The CD34 molecule is expressed on virtually all hematopoietic precursor cells (12, 13), including the multipotent stem cells (14). The CD34 molecule is the earliest marker in human for precursors of colony forming cells in the bone marrow (8,9).

Since CD34 is transiently expressed during hematopoiesis, the average of CD34 positive cells is very low in healthy donor hematologic specimens: CD34 antigen is expressed on 1% to 5% of bone marrow cells or cord blood cells (12), while in peripheral blood, only 0.1% to 0.5% of the cells are CD34 positive (15).

This low frequency can be greatly increased in peripheral blood sample from patients recovering from aplasia or under recombinant cytokine treatment for mobilization of CD34+ cells for apheresis (16,17).

The CD34 glycoprotein is not restricted to hematopoietic progenitors (18) and has been detected on capillary endothelial cells (18,19), and on bone marrow stromal cells and their precursors (20).

This 581 antibody has been assigned to the CD34 cluster of differentiation at the Vth International Workshop on Human Leukocyte Differentiation Antigens in Boston (1993).

REAGENT

Clone 581
Isotype IgG1 mouse
Immunogen Human CD34+ leukemia cells
Hybridoma NS0 x Balb/c spleen cells
Source Ascites fluid
Purification Ion exchange or affinity chromatography
Conjugation PE: R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of IgG.
Excitation wavelength: 488 nm
Maximum emission wavelength: 575 nm
Main emission color: Orange-red
Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

Studies (1,2,3,21) indicate that CD34 may be useful in the following applications:

The CD34 antigen is expressed in certain cases of acute leukemia, both in lymphoid and in myeloid leukemias. CD34 antibodies can help delineate B-cell lymphoma from acute lymphoblastic leukemia. CD34 antibodies may be used in some instances of chronic myeloid leukemia (CML), where increased numbers of CD34+ can be noticed.

CD34 antibodies may be used for qualitative and quantitative assessments of bone marrow or blood sample hematopoietic progenitor cells (22,23).

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2-8 C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 20 - 25 C prior to use.

PROCEDURE

DIRECT LABELLING OF WHOLE BLOOD OR BONE-MARROW WITH CONJUGATED MONOCLONAL ANTIBODY FOLLOWED BY RED BLOOD CELL LYSING METHOD.

Preliminary remarks:

In the following procedure, we recommend to use an ammonium chloride-based lysing solution with satisfactory results, provided that the sample is thoroughly washed with phosphate-buffered saline.

Procedure:

1. Pipet 100 µL of specimen into two tubes (control and test).
 2. Add 30 µL of blocking mouse Ig at 300 µg/mL (optional).
 3. Add 20 µL CD34-PE to the test tube or 20 µL of conjugated isotypic control to the control tube.
 4. Mix gently and incubate for 15 min. at room temperature (18-25 C) in the dark.
 5. Wash the preparation by adding 3 mL of PBS.
 6. Centrifuge at 300 x g for 10 min. at room temperature.
 7. Discard the supernatant and resuspend the pellet in 100 µL of PBS.
 8. Lyse the stained specimen, according to the manufacturer's recommendations.
 9. Wash the preparation by fulfilling the tubes with PBS.
 10. Centrifuge at 300 x g for 10 min. at room temperature.
 11. Discard the supernatant and resuspend the pellet in 1 mL of PBS + 0.5% formaldehyde.
- The preparations are ready to be analyzed by flow cytometry within 2 hours.
(Keep the preparation at 2 to 8 C for an analysis within 24 hours.)

DIRECT LABELLING OF ISOLATED MONONUCLEAR CELLS OR CULTURED CELLS.

After isolation of mononuclear cells (MNC) by density gradient centrifugation:

resuspend cells in PBS containing 0.5% BSA and 0.05% NaN₃; count the viable cells (Trypan Blue test), and adjust the cell concentration to 5 x 10⁶ to 1 x 10⁷ cells/mL.

Cell culture adjustment:

1871EX250198 01/02/98 AC-97256



COULTER

PARTNERS IN CELL ANALYSIS

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Resuspend the pellet in PBS containing 0.5% BSA and 0.05% NaN₃ and adjust cell concentration to 5 x 10⁶ to 1 x 10⁷ cells/mL.

Procedure for direct immunolabelling:

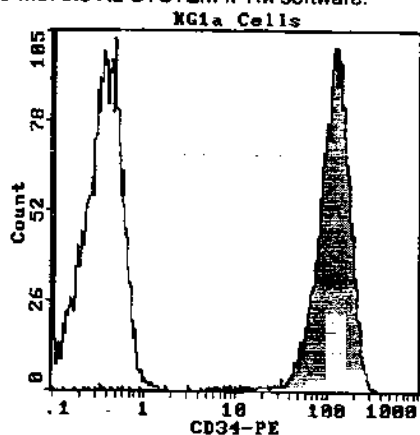
1. Pipet 100 µL of the suspension into two tubes (test and control).
2. Add 30 µL of blocking mouse Ig at 300 µg/mL (optional).
3. Add 20 µL of CD34-PE to the test tube, or 20 µL of conjugated isotypic control to the control tube.
4. Mix gently and incubate during 30 min. at 2-8 C in the dark.
5. Wash the preparation by adding 3 mL of cold (2-8 C) PBS containing 0.5% BSA and 0.05% NaN₃.
6. Centrifuge at 300 x g for 5 min. at 2-8 C.
7. Repeat steps number 5 and 6 two times.
8. Resuspend stained pellets in 1 mL of cold (2-8 C) PBS. Analyze by flow cytometry within 2 hours.

(Cells can be fixed in PBS containing 0.5% formaldehyde, and kept at 2-8 C for analysis within 24 hours.)

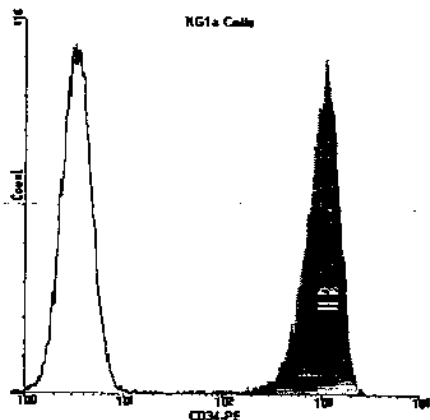
EXAMPLE DATA

The histograms below are monoparametric representations (Count versus Fluorescence Intensity) of KG-1a cell line. Staining is with CD34-PE monoclonal antibody (PN IM1871). The isotypic control labeling (PN IM0670) is shown in light.

Acquisition is with a COULTER R EPICS R XL TM flow cytometer. Analysis is with the XL SYSTEM II TM software.



Acquisition is with a Becton Dickinson FACScan TM flow cytometer. Analysis is with the LYSYS II TM software.



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