IOTest® CD34-PE

PN IM1420 - 100 tests - 20 µL/test – Liquid – Clone Immu133

For Research Use only. Not for use in diagnostic procedures

SPECIFICITY

The CD34 glycoprotein is a transmembrane single chain molecule. Its molecular weight is about 110 kDa. The extracellular domain is heavily N- and O-glycosylated (1 – 4) and the cytoplasmic sequence reveals two sites for activated protein kinase C phosphorylation and one site for tyrosine phosphorylation (3). This antigen is the earliest known marker in human for hematopoietic progenitor cells (5, 6).
There are three classes of CD34 epitopes defined by differential sensitivity to enzymatic cleavage with glycoprotease from Pasteurella haemolytica, with chymopapain and with neuraminidase.
The Immu133 monoclonal antibody recognizes specifically a Class I epitope, neuraminidase sensitive, and glycoprotease sensitive which is regularly expressed on normal samples.
The CD34 molecule is expressed on virtually all hematopoietic precursor cells (7), including the multipotent stem cells (8). The CD34 molecule is the earliest marker in human for precursors of colony forming cells in the bone marrow (5, 6).
The CD34 glycoprotein is not restricted to hematopoietic progenitors (9) and has been detected on capillary endothelial cells (9 – 11), and on bone marrow stromal cells and their precursors (12).
The Immu133 monoclonal antibody has been assigned to the CD34 cluster of differentiation at the Vth International Workshop on Human Leukocyte Differentiation Antigens in Boston, USA, in 1993 (13).

REAGENT

Monoclonal Antibody CD34-PE
PN IM1420 – 100 tests - Liquid – 20 µL/test

Clone	Immu133
Isotype	IgG1 Mouse
Immunogen	KG1a cell line
Hybridoma	P3-X63-Ag.8.653 myeloma x Balb/c spleen cells
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography
Conjugation	R-phycoerythrin (PE)
Molar Ratio	PE/Ig : 0,5 – 1,5
Fluorescence	Excites at 488 nm Emits at 575 nm.

REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline pH 7.4, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin.

APPLICATION

Flow cytometry.

STATEMENTS OF WARNING:

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 they might transmit 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.
7. Use good laboratory practises when handling this reagent.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2-8 °C in the dark.Do not freeze.

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 labeling 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% NaN_3; count the viable cells (Trypan Blue test), and adjust the cell concentration to 5×10^6 to 1×10^7 cells/mL.
Cell culture adjustment:
Resuspend the pellet in PBS containing 0.5% BSA and 0.05% NaN_3 and adjust cell concentration to 5×10^6 to 1×10^7 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% NaN_3.
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. The isotypic control labeling (See catalog for PN) is shown in light.
Figure 1:

IOTest® CD34-PE

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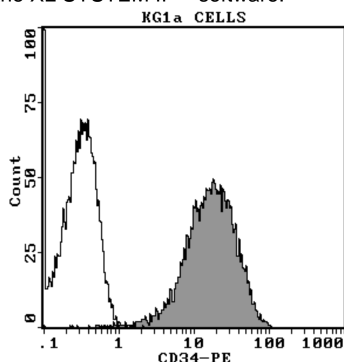
Acquisition is with a COULTER ® EPICS ® XL ™ flow cytometer. Analysis is with the XL SYSTEM II ™ software.



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