Instructions for use

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PeliCluster CD41a

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Research use only

Characteristics

Monoclonal mouse antibody.
Clone: CLB-tromb/7, 6C9.
This clone has been derived from hybridization of SP2/0 cells with spleen cells of a BALB/c mouse immunized with human platelets. The antibody was submitted to CD41a in the Third, Fourth and Sixth International Workshop on Human Leukocyte Differentiation Antigens.
Isotype: Mouse IgG1.
Source: Culture supernatant purified by affinity chromatography.
Apparent molecular mass under reducing conditions: GPIIIa:110 kDa; GPIIb alpha chain:125 kDa, beta chain: 25 kDa.
The monoclonal mouse antibody reacts with megakaryocytes, platelets and recognize the intact thrombocyte GPIIb/IIIa complex, i.e. it does not bind to dissociated GPIIb or GPIIIa. The monoclonal mouse antibody does not bind or reduced to thrombocytes from patients with Glanzmann's thrombasthenia and do not recognize the vitronectin receptor, which contains GPIIa as a beta subunit, cf. CD61 (1).

Package contents

Each vial contains 1 mL with approximately 0.2 mg/mL monoclonal mouse antibody and 10 mg BSA in 20 mM TRIS and 150 mM NaCl, pH 8.0. 200 test/vial.
Preservative: Sodium azide 0.1% (w/v).

Precautions

Leaking or damaged vials must not be used. The reagent cannot be assumed to be free from infectious agents. Care must be taken in the use and disposal of each container and its contents. Waste-disposal, after completion of the test, should be performed according to your laboratory regulations.
Monoclonal mouse antibodies should be stored in the dark at 2-8°C.
The reagent is stable until the expiry date stated on the vial label.

Application

The monoclonal mouse antibody is a useful marker for studies of megakaryoblasts and megakaryoblastic leukaemias.
Method: Indirect immunofluorescence staining with analysis by flow cytometry or fluorescence microscopy.

Reagents and Equipment

- **Conjugate**:
- FITC conjugated Goat anti-Mouse immunoglobulin antiserum, solid phase adsorbed with human immunoglobulins or comparable product. Remove aggregates by centrifugation at 1000 x g for 10 minutes. Dilute as mentioned in the procedure.
- **Wash- and dilution buffer for mononuclear cells,(PBS/BSA)**: Phosphate Buffered Saline containing 0.2% BSA (w/v).
- **Wash- and dilution buffer for platelets, (Seq).**
  Sequestrine buffer, storage 1 month at 2-8°C.
  10 x stock solution, dissolve in 500 mL distilled water:
  Na2HPO4.2H2O: 15.65 g
  Na2EDTA.2H2O: 16.65 g (Complexon)
  NaCl: 45.0 g
  Prior to use, dilute in distilled water, add BSA till final concentration of 0.2% (w/v). Mix and adjust pH to 6.8.
- **Permeabilisation buffer, (BFA):**
  Buffered Formaldehyde Acetone, storage 1 month at 2-8°C.
  Dissolve in 450 mL distilled water:
  Na2HPO4.2H2O: 300 mg
  KH2PO4 : 1500 mg
  Add to 30 mL of this buffer 45 mL acetone and 25 mL formaldehyde (37%), mix and adjust pH to 6.8 – 7.0.
- **Fixation buffer, PFA/BSA:**
  Para-Formaldehyde 1% in PBS, containing 0.2% BSA (pH 7.2).
- **Lysing solution**
- **Embedding medium:**
  70% glycerol in PBS.
- **Microwell plates and tubes:** Microwell plates (96 wells, V bottom) or plastic flowcytometry tubes.

Cell isolation.

1. Isolation of mononuclear cells.

1.1 Draw blood into a blood collecting tube containing EDTA, store at room temperature (18-25°C).
1.2 Isolate the mononuclear cells, preferably within 4 hours after collection, by standard density gradient centrifugation (e.g. Ficoll-Paque, Pharmacia).

1.3 Wash cells at least twice with PBS/BSA and adjust cell concentration to 1x107 cells/mL in PBS/BSA.

2. Isolation of platelets.

2.1 Draw blood into a collecting tube containing EDTA. (Care should be taken to prevent activation of platelets. The expression of most platelet activation markers is maximal after stimulation with 1 U/mL thrombin).

2.2 Prepare Platelet Rich Plasma (PRP) by centrifugation of EDTA-anticoagulated blood at 500 x g for 5 minutes, use no brake, transfer supernatant (PRP) with a plastic pipette into a 50 mL conical plastic centrifuge tube.

2.3 Wash by filling the tube with Seq, mix and centrifuge at 1600 x g for 10 minutes. Discard the supernatant and repeat once more. If erythrocytes are visible in the pellet after the first washing, lyse the erythrocytes by adding 5 mL NH_4Cl for 10 minutes in an ice-water bath , then continue with the last wash.

2.4 Adjust the platelet concentration to 1x108 cells/mL in Seq. buffer.

Procedures.

1. Mononuclear cell membrane flow cytometry / microscopy.

1.1 Transfer 45 µL of the mononuclear cell suspension into microwell plate or tubes and add 5 µL monoclonal antibody*. Mix gently and incubate for 30 minutes at 2-8°C.

1.2 Wash by mixing and adding PBS/BSA to the microwell plate (1st wash 150 mL, 2nd wash 200 mL) or tubes (2 mL), centrifuge at 500 x g for 5 minutes and aspirate the supernatant. Repeat this procedure once more.

1.3 Add 50 µL conjugate, diluted 1:80 in PBS/BSA. Mix and incubate for 30 minutes at 2-8°C in the dark.

1.4 Wash: see step 1.2.

1.5 Prepare cells for analysis: For flow cytometry resuspend the cells by adding 200 µL PFA/BSA to the microwell plates or tubes. If a microwell plate was used, the contents are transferred to appropriate tubes. For fluorescence microscopy, resuspend the cells in 50 µL embedding medium, transfer cells to a microscope slide and place a cover glass.

2. Mononuclear cell cytoplasmic flow cytometry (1).

2.1 For 20 tests, resuspend 1x107 mononuclear cells in 250 Ll of BFA and incubate for only 2 seconds at 18-25°C. (This incubation will stop by the addition of the wash buffer, a longer BFA fixation will diminish the fluorescence intensity).

2.2 The cells are immediately washed by mixing and adding PBS (2-8°C). Centrifuge at 500 x g for 5 minutes, repeat this procedure once more. Use 5x105 cells per test.

2.3 Transfer 45 µL of the cell suspension into microwell plate or tubes and add 5 µL monoclonal antibody*. Mix gently and incubate for 30 minutes at 2-8°C.

2.4 Wash by mixing and adding PBS/BSA to the microwell plate (1st wash 150 mL, 2nd wash 200 mL) or tubes (2 mL), centrifuge at 500 x g for 5 minutes and aspirate the supernatant, repeat this procedure once more.

2.5 Add 50 µL conjugate, diluted 1:80 in PBS/BSA. Mix and incubate for 30 minutes at 2-8°C in the dark.

2.6 Wash: see step 2.2.

2.7 For analysis, the cells are resuspended in 200 µL PBS/BSA, if a microwell plate was used, the contents are transferred to appropriate tubes.

3. Mononuclear cell cytoplasmic fluorescence microscopy.

3.1 Adjust cell concentration to 2x106 cells/mL.

3.2 Prepare slides by briefly centrifuging 50 µL PBS/BSA at maximum speed in a cytocentrifuge, next centrifuge 50 µL cell suspension for 5 minutes at 500 g.

3.3 Allow the slides to dry for 30 minutes at 18-25°C.

3.4 Fix the cells in a slide jar containing acetone for 10 minutes at 18-25°C.

3.5 Wash the slides in a slide jar containing PBS under continuous gentle agitation for 3 separate 5 minutes changes. Carefully dry the slides around the spot.

3.6 Dispense 50 µL diluted monoclonal mouse antibody* over the spot. Incubate for 30 minutes at 18-25°C in a humid dark container.

3.7 Wash the slides: see step 3.5.

3.8 Dispense 50 µL conjugate, diluted 1:160 in PBS, over the spot and incubate for 30 minutes at 18-25°C in a humid dark container.

3.9 Wash the slides: see step 3.5.

3.10 Dispense one drop of embedding medium over the spot and place a cover glass.

4. Platelet membrane flow cytometry / microscopy.

4.1 Transfer 45 µL of platelet suspension into the microwell plate or tubes and add 5 µL monoclonal antibody*. Mix gently and incubate for 30 minutes at 2-8°C.

4.2 Wash by mixing and adding Seq. buffer to the microwell plate (1st wash 150 mL, 2nd wash 200 mL) or tubes (2 mL). Centrifuge at 1000 x g for 5 minutes and aspirate the supernatant, repeat this procedure once more.

4.3 Add 50 µL conjugate, diluted 1:80 in Seq. Mix and incubate for 30 minutes at 2-8°C in the dark.

4.4 Wash: see step 4.2.

4.5 Prepare cells for analysis:
For flow cytometry, resuspend the cells by adding 200 µL Seq to the microwell plate or tubes. If a microwell plate was used the contents are transferred to appropriate tubes.
For fluorescence microscopy, resuspend the cells in 50 µL embedding medium, transfer cells to a microscope slide and place a cover glass.

Note:

* In general, 5 µL undiluted monoclonal mouse antibody can be used. Alternatively an optimal dilution can be determined. To determine background fluorescence always use a negative control from the same isotype.

References

1 Modderman, P.W. et al., Thrombosis and Haemostasis, 60, 68 (1988).

2 Slaper-Cortenbach, I.C.M. et al., The flow-cytometric detection of Terminal deoxynucleotidyl Transferase (TdT) and other intracellular antigens in combination with membrane antigens in acute lymphatic leukaemia's, Blood, 72 (1988), 1639-1644.