

Product No. F-7777
Lot 074H4841

Monoclonal Anti-Human CD42a
FITC Conjugate
Purified Mouse Immunoglobulin
Clone BL-H6

Monoclonal Anti-Human CD42a (mouse IgG1 isotype) is derived from the BL-H6 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with human lymphocytes. The isotype is determined using Sigma Immunotype™ Kit (Sigma Stock No. ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD42a monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The conjugate is provided (200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Description

The CD42a (GPIX) antigen^{2,3} is a single chain 23 kD membrane glycoprotein that forms a non-covalent complex with GPIb (CD42b). This complex is known as the receptor for ristocetin-induced binding by von Willebrand factor and also as von Willebrand factor-dependent adhesion receptor. The complex is a major component of the platelet surface. Binding of the GPIb α chain to von Willebrand factor bound to the exposed subendothelium of damaged vascular walls is essential for platelet adhesion. CD42a is expressed on platelets and megakaryocytes. It is reduced or absent in platelets from patients with Bernard-Soulier syndrome. The CD42a antigen is down-regulated on platelet activation. FITC Monoclonal Anti-Human CD42a recognizes the human CD42a antigen expressed on platelets and megakaryocytes. The antibody immunoprecipitates non-reduced GPIb/IX and reacts with non-reduced GPIX in immunoblotting assays. Besides staining platelets and megakaryocytes, the antibody may stain platelet sequestering cells. The antibody also immobilizes immune complexes of GPIb/IX and human GPIb autoantibodies. The epitope recognized by the antibody is sensitive to routine formalin-fixation and paraffin-embedding.

Performance

When assayed by flow cytometric analysis, using 10 µl of the antibody to stain 1×10^6 cells, 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.

F/P Molar Ratio: 3.0**Uses**

Monoclonal Anti-Human CD42a may be used for:

1. Detection and enumeration of platelets in health and disease.
2. Detection of human platelet autoantibodies.
3. Identification of megakaryoblasts and megakaryocytes in frozen tissue sections.
4. Typing of leukemias.
5. Studies of Bernard-Soulier syndrome.

Storage

Store at 0-5 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

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

Note: In order to obtain best results in different preparations, it is recommended that each individual user determine their optimum working dilutions by titration assay.

Procedure for Direct Immunofluorescent Staining of Platelets

Reagents and Materials Needed but not Supplied

1. Human peripheral blood platelets isolated from platelet rich plasma.
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4 containing 1% BSA, 0.1% NaN₃.
3. FITC conjugated, isotype matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. F-6397).
4. 12x75mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma Product No. T-0776), 0.2% in 0.01 M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Flow cytometer or fluorescent microscope.

Procedure

1. Prepare platelet suspension in plasma by centrifuging whole anticoagulated blood at 160 x g for 15 minutes at room temperature or use washed platelet suspension. Adjust cell suspension to 1 x 10⁷ cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g. trypan blue). For each sample, add 100 µl or 1 x 10⁶ cells per tube.
2. Add 10 µl of conjugate to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 - 22°C) for 30 minutes. Protect from light at this and all subsequent steps.
Proper controls to be included for each sample:
 - a. An autofluorescence control: 10 µl diluent in place of conjugated antibody followed by steps 3-8.
 - b. A negative staining control: 10 µl of FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (Sigma Product No. F-6397) at the same concentration as test antibody, followed by steps 3-8.
3. Add 2 ml of diluent to all tubes.
4. Pellet cells by centrifugation at 1,000 x g for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml diluent.
7. Pellet cells as in step 4 and repeat washing procedure (steps 3-5) once.
8. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-

matched to the antibody and of the same concentration and F/P molar ratio as the 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.

References

1. Leucocyte Typing IV, Knapp, W., et al., (eds.), Oxford University Press, pp. 955, 976-983, 1002 (No. P006) (1989).
2. Du, X., et al., Blood, **69**, 1524 (1988).
3. Barclay, A., et al., The Leucocyte Antigen Facts Book, Academic Press, San Diego, CA, p. 192 (1993).

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