

MONOCLONAL ANTIBODIES TO HUMAN LEUKOCYTE ANTIGENS



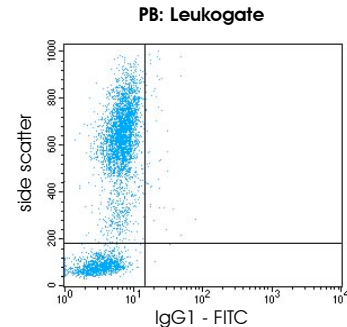
IgG1 ANTIBODY Negative Control Reagent

Available Forms:

Purified Antibody	Cat. No.: GM-4991	2ml	0,2mg
Fluorescein Conjugate	Cat. No.: GM-4992	2ml	100 Tests
Phycoerythrin Conjugate	Cat. No.: GM-4993	2ml	100 Tests

Specification

Specificity (Synonyms):	Negative Control
Clone:	VI-AP
Immunogl. Class:	IgG1
Species:	Mouse
Purification:	Chromatography
Fluorochrome:	un-coupled, FITC or PE conjugated
Storage Buffer:	PBS pH 7.2, 1% BSA, 0.05% NaN ₃



Introduction

This ready to use Negative Control reagent contains purified, fluorescein or phycoerythrin conjugated mouse immunoglobulin molecules of IgG1 isotype, which have been selected on the basis of their binding characteristics: no specific binding to human cell surface or intracellular antigens, same low range of nonspecific binding to human leukocytes as other AN DER GRUB Reagents.

Intended use

This isotype control IgG1 is suitable as a Negative Control to be used in combination with AN DER GRUB reagents for the:

- Enumeration of Myeloid Cells
- Analysis of Myeloid Differentiation Stage
- Enumeration of B-cells and Precursors
- Enumeration of T-cells and Precursors
- Analysis of Leukemia Cells
- Analysis of Immunodeficiency States

The Negative Control reagent permits to estimate the degree of non-specific binding of isotype matched immunoglobulins to leukocytes via e.g. Fc-receptors. It enables the expert to set flow cytometric parameters accordingly.

Results must be put within the context of other diagnostic tests as well as the clinical history of the patient by a certified professional before final interpretation.

Specificity

The clone VI-AP reacts with calf intestine alkaline phosphatase and does not show cross-reactivity with human proteins.

Storage

AN DER GRUB monoclonal antibody reagents contain optimal concentrations of affinity-purified antibody. For stability reasons this monoclonal antibody solution contains sodium azide. These reagents should be stored at 2-8°C (DO NOT FREEZE!) and protected from prolonged exposure to light. Stability of the reagent: Please refer to the expiry date printed onto the vial. The use of the reagent after the expiration date is not recommended.

Samples

Biological fluids (blood, bone marrow, and others) must be collected under sterile conditions. Anticoagulation with EDTA or heparin is recommended. The samples should be stored at room temperature until used. For optimal results, samples should be processed and analyzed within 24 hours.

Samples with high numbers of non-viable cells might cause false results, such cases require determination of cell viability with e.g. propidium iodide.

All biological samples have to be handled with caution. Always consider them as potentially infective. Use appropriate precautions such as gloves, lab-coat, etc.

Staining Procedure

Direct Immunofluorescence (Staining Procedure)

ADG fluorochrome labeled antibodies are designed for use with either whole blood or isolated mononuclear cell (MNC) preparations.

Proposed staining procedure for whole blood in short:

- For each sample add 50 μ l of EDTA anti-coagulated blood to a 3-5 ml tube
- Add 20 μ l of the appropriate AN DER GRUB monoclonal antibody conjugate
- Incubate the tube for 15 minutes at 4°C or at room temperature in the dark
- Add 100 μ l ADG-LYSE (Cat.No. GAS-003) to each tube and incubate for 10 minutes at room temperature
- Add 3-4 ml of distilled water and vortex, incubate for 5-10 minutes at room temperature
- Centrifuge tube for 5 minutes at 300 g
- Aspirate supernatant and resuspend pellet in 0.3 ml of sheath fluid
- Analyze immediately or store samples at 2-8°C in the dark and analyze within 24 hours

For "No-Wash" protocol please refer to www.andergrub.com

Proposed staining procedure for MNC in short:

- Carefully add 20 μ l antibody conjugate and 50-100 μ l MNC to the bottom of a tube
- Vortex at low speed for 1-2 seconds
- Incubate for 15-30 minutes at 2-8°C or at room temperature
- Centrifuge tubes for 5 minutes at 300 g
- Remove supernatant, resuspend cells in 2-5 ml of phosphate buffered saline (PBS) and centrifuge cells again for 5 minutes at 300 g
- Remove supernatant and resuspend cells in sheath fluid for immediate analysis or resuspend cells in 0.5 ml 1% formaldehyde and store them at 2-8°C in the dark. Analyze fixed cells within 24 hours

Indirect Immunofluorescence (Staining Procedure)

- Mix 20 μ l AN DER GRUB purified antibody with 50 μ l whole blood or MNC suspension
- Incubate for 15 minutes at 2-8°C
- Wash cells with phosphate buffered saline (PBS)

- Add to cell pellet 20 μ l of affinity purified, fluorochrome labeled F(ab)₂ anti mouse Ig antibodies
- Incubate for 15 minutes at 2-8°C
- Wash cells with phosphate buffered saline (PBS) or proceed as described for direct staining

Sensitivity

The sensitivity of VI-AP mAb is determined by staining well-defined blood samples from representative donors with serial-fold mAb dilutions to obtain a titration curve that allows relating the mAb concentration to the percentage of stained cells and geometric MFI (mean fluorescence intensity). For this purpose, a mAb-concentration range is selected to include both the saturation point (i.e. the mAb dilution expected to bind all epitopes on the target cell) and the detection threshold (i.e. the mAb dilution expected to represent the least amount of mAb needed to detect an identical percentage of cells). In practice, 50 μ l of leukocytes containing 10⁷ cells/ml are stained with 20 μ l mAb of various dilutions to obtain a titration curve and to identify the saturation point and detection threshold. The final concentration of the product is then adjusted to be at least 3-fold above the detection threshold. In addition and to control lot-to-lot variation, the given lot is compared and adjusted to fluorescence standards with defined intensity.

Limitations of the technique

Flow cytometry should be performed by professional users only. Improper alignment of the flow cytometer, inaccurate

compensation of fluorescence leaking into other channels as well as incorrect positioning of regions may lead to false results. Lysis of red cells might be impossible for various reasons. In such instances it is recommended to isolate mononuclear cells (MNC) via density gradient centrifugation prior to staining. Results will be correct and reproducible as long as the procedures used respect the technical recommendations and obey good laboratory practice. The properties of this mAb have been determined using EDTA anti-coagulated peripheral blood.

Precautions

For professional users only.

This reagent contains sodium azide. To avoid the development of hazardous conditions, reagents containing azide should be diluted in running water prior to be discarded. Similar to the work with other biological products, proper handling procedures are recommended.

Warranty

The products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, that extend beyond the description on the label of the product. ADG's sole liability is limited to either replacement of the products or refund of the purchase price. ADG is not liable for property damage, personal injury, or economic loss caused by the product.

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