

MONOCLONAL ANTIBODIES TO HUMAN LEUKOCYTE ANTIGENS



TCR α/β ANTIBODY

Available Form (Reagent provided):

Phycoerythrin Conjugate

Cat. No.: GM-4183

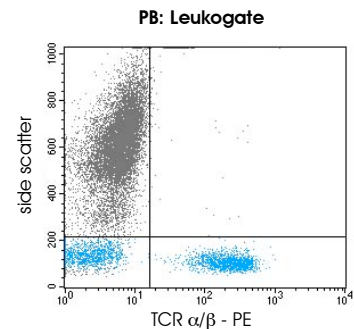
2ml

100 Tests



Specification

Specificity (Synonyms):	T-cell receptor alpha/beta complex
Clone:	BMA 031
Immunogl. Class:	IgG2b
Species:	Mouse
Purification:	Chromatography
Fluorochrome:	PE
Storage Buffer:	PBS pH7.2, 1 mg/ml BSA, 0.05% NaN ₃



Introduction

T-lymphocytes recognize foreign antigens via their clonotypic T-cell receptor (TCR). The TCR consists of two disulfide-linked TCR chains, which are embedded in a sheath of CD3 molecules, commonly referred as the TCR/CD3 complex. Among TCR heterodimers two forms can be distinguished: α/β TCRs and γ/δ TCRs. T-lymphocytes expressing the α/β TCR make up the majority of T-cells in peripheral blood and bone marrow and can be distinguished from γ/δ T-lymphocytes expressing the γ/δ TCR. In normal T-cell development α/β TCR can first be detected in the cortical thymocyte stage. A variety of studies have demonstrated the usefulness and reliability of anti-TCR mAbs for the classification of acute T-cell leukemias (T-ALL). In T-ALL surface α/β TCR expression is characteristic for the T-ALL IVa subtype according to the EGIL classification.

Intended use

MAb BMA 031 binds to T-lymphocytes expressing the α/β TCR/CD3 complex. The α/β T-cell receptor (TCR) is the specific antigen receptor of the majority of human T-lymphocytes. It is expressed on thymocytes and peripheral T cells, which don't express the γ/δ TCR. The α/β TCR mAb permits the identification and enumeration of normal and leukemic T-cell populations in human biological samples (blood, bone marrow and others) using flow cytometry.

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. Analyses performed with this antibody should be paralleled by positive and negative controls. If unexpected results are obtained, which cannot be attributed to differences in laboratory procedures, please contact us.

Specificity

Antibody BMA 031 reacts with a constant determinant expressed on the surface of all α/β TCR expressing T-lymphocytes.

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 this 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)

AN DER GRUB 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

Sensitivity

The sensitivity of the α/β TCR 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, 50µl of leukocytes containing 10⁷ cells/ml are stained with 20µl 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 antibody is provided in a concentration that will allow to unequivocally detect specific cells. It is therefore strongly recommended to stick to the staining protocol in terms of concentration and volume regarding cells and antibody.

The therapeutic use of antibodies might influence the recognition of target-antigens by this antibody. The reaction pattern of αβ TCR mAb alone is not sufficient to diagnose "leukemia". Combination with other antibodies in multi-color stainings is strongly recommended. 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.

For In Vitro Diagnostic Use

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.

Selected References

- Bene, M. C., Castoldi, G., Knapp, W., Ludwig, W. D., Matutes, E., Orfao, A. & van't Veer, M. B. (1995) *Leukemia* **9**, 1783-6.
- Borst, J., van Dongen, J. J., de Vries, E., Comans-Bitter, W. M., van Tol, M. J., Vossen, J. M. & Kurlle, R. (1990) *Hum Immunol* **29**, 175-88.
- Davodeau, F., Peyrat, M. A., Romagne, F., Necker, A., Hallet, M. M., Vie, H. & Bonneville, M. (1995) *J Exp Med* **181**, 1391-8.
- Janssen, O., Wesselborg, S., Heckl-Ostreicher, B., Pechhold, K., Bender, A., Schondelmaier, S., Moldenhauer, G. & Kabelitz, D. (1991) *J Immunol* **146**, 35-9.
- Kabelitz, D., Bender, A., Schondelmaier, S., da Silva Lobo, M. L. & Janssen, O. (1990) *J Immunol* **145**, 2827-32.
- Knight, R. J., Kurlle, R., McClain, J., Racenberg, J., Baghdasarian, V., Kerman, R., Lewis, R., van Buren, C. T. & Kahan, B. D. (1994) *Transplantation* **57**, 1581-8.
- Miossec, C., Faure, F., Ferradini, L., Roman-Roman, S., Jitsukawa, S., Ferrini, S., Moretta, A., Triebel, F. & Hercend, T. (1990) *J Exp Med* **171**, 1171-88.
- Nashan, B., Wonigeit, K., Schwinzer, R., Schlitt, H. J., Kurlle, R. & Pichlmayr, R. (1987) *Transplant Proc* **19**, 4270-2.
- Peyrat, M. A., Davodeau, F., Houde, I., Romagne, F., Necker, A., Legeŕ, C., Cervoni, J. P., Cerf-Bensussan, N., Vie, H., Bonneville, M. & et al. (1995) *J Immunol* **155**, 3060-7.
- Schwinzer, R., Schlitt, H. J. & Wonigeit, K. (1992) *Cell Immunol* **140**, 31-41.
- Thibault, G. & Bardos, P. (1995) *J Immunol* **154**, 3814-20.

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Explanation of symbols

REF	Catalog number
IVD	In vitro diagnostic medical device
①	Consult instructions for use
2° -8°	Temperature limitation
☀	Keep away from sunlight
LOT	Batch code
⌚	Use by
⚠	Contains sufficient for (N) Tests
🏭	Manufacturer