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Diagnostik & molekulare Diagnostik



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Mouse anti CD3, conjugated to FITC

nordicmubio.com/products/Mouse-anti-CD3-conjugated-to-FITC/GM-4012-CE_slash_IVD

Catalog number: **GM-4012-CE/IVD**

Clone	UCHT1
Isotype	IgG1
Product Type	Primary Antibodies
Units	2ml (100 Tests)
Host	Mouse
Species Reactivity	Human
Application	Flow Cytometry Immunofluorescence

Background

UCHT1 is directed against human CD3 – the multichain complex associated with the T-cell receptor. Precursor T-cells are surface CD3 negative but positive for cytoplasmic CD3. All mature T-cells are both cytoplasmic and surface CD3 positive. The UCHT1 antibody permits the identification and enumeration of normal and leukemic human blood and bone marrow cells 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.

Product

2 ml of FITC-conjugated anti CD3 (clone UCHT1) in PBS pH 7.2, 1% BSA, and 0.05% NaN₃, approximately 100 tests.

Product Form: FITC

Formulation: PBS pH 7.2, 1% BSA, 0.05% NaN₃

Purification Method: Purified by Chromatography

Specificity

The CD3 mAb (clone UCHT1) recognizes cytoplasmic CD3 epsilon in precursor T-cells and cytoplasmic and surface CD3 epsilon in mature T-lymphocytes. The sensitivity of UCHT1 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). The sensitivity of UCHT1 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). In practice, 50 µl of leukocytes containing 10⁷ cells/ml are stained with 20 µl 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

Applications

Staining Procedure for Surface CD3: Direct Immunofluorescence (Staining Procedure)

Nordic-MUBio 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 µl of EDTA anti-coagulated blood to a 3-5 ml tube - Add 20 µl of the appropriate Nordic-MUBio monoclonal antibody conjugate - Incubate the tube for 15 minutes at 4°C or at room temperature in the dark - Add 100 µl Nordic-MUBio-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.nordicmubio.com Proposed staining procedure for MNC in short: - Carefully add 20 µl antibody conjugate and 50-100 µl 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 µl Nordic-MUBio purified antibody with 50 µl 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 µl 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 Staining Procedure for Cytoplasmic CD3: Permeabilization and Staining Procedure - In combination with our Permeabilization Kit FIX&PERM® (Cat. No. GAS-002) intracellular CD3 can be easily stained in cell suspensions. - For each sample to be analyzed add 50 µl of whole blood, bone marrow

or mononuclear cell suspension in a 5ml tube - Add 100 µl of Reagent A (Fixation Medium, stored and used at room temperature) - Incubate for 15 minutes at room temperature - Add 5ml phosphate buffered saline and centrifuge cells for 5 minutes at 300 g - Remove supernatant and add to cell pellet 100 µl Reagent B (Permeabilization Medium) and 20 µl of the CD3 monoclonal antibody conjugate - Vortex at low speed for 1-2 seconds - Incubate for 15 minutes at room temperature - Wash cells with phosphate buffered saline as described above - Remove supernatant and resuspend cells in sheath fluid for immediate analysis or resuspend cells in 0.5 ml 1.0 % formaldehyde and store them at 2- 8°C in the dark. - Analyze fixed cells within 24 hours.

Storage

Nordic-MUBio 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. If a slight precipitation occurs upon storage, this should be removed by centrifugation. It will not affect the performance or the concentration of the product. 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.

Caution

When used for in vitro diagnostic purposes 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. This product may contain hazardous ingredients. Please refer to the Safety Data Sheets (SDS) for additional information and proper handling procedures. Dispose product remainders according to local regulations. This datasheet is as accurate as reasonably achievable, but Exalpha Biologicals accepts no liability for any inaccuracies or omissions in this information.

References

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