3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Monoclonal Anti-CD3- Quantum Red™
clone UCHT-1
produced in mouse, purified immunoglobulin

Catalog Number **R9011**

Product Description

Monoclonal Anti-CD3 (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cell line NS-1 and splenocytes from Balb/c mice immunized with human thymocytes followed by Sezary T cells. The isotype is determined by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents , Catalog Number ISO2. The product is prepared by conjugation of Quantum Red with purified CD3 monoclonal antibody. Quantum Red is Sigma's tandem fluorochrome in which a small organic dye, Cy5, is covalently linked to R-Phycoerythrin (PE). The PE absorbs light energy at 488 nm and emits in the excitation range of Cy5 which acts as the acceptor dye. The complex then emits at 670 nm. The conjugate is purified by gel filtration to remove unbound Quantum Red and antibody. No free Quantum Red or free antibody is detectable.

Monoclonal Anti-CD3 recognizes the CD3 complex which is composed of 5 chains designated γ, δ ε, ξ and η having a molecular mass distribution of 16, 20, and 25 - 28 kDa. The CD3 human lymphocyte surface antigen is a glycoprotein thought to be associated with the T cell antigen receptor and to be involved in transmission of activation signals. The CD3 antigen is present on 60-80% of normal peripheral blood mononuclear cells, 20-40% of normal spleen cells, 40% of normal thymocytes, the majority of T-CLL and approximately 70% of T-ALL. The antibody stains the cytoplasm of cerebellar Purkinje cells but does not stain B lymphocytes, monocytes, granulocytes, or NK cells. The epitope recognized by this clone is sensitive to routine formalin fixation and paraffin embedding. Cryostat sections post-fixed in formalin can also be stained.

Reagents

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide as a preservative.

Product Profile

Quantum Red Conjugated Monoclonal Anti-Human CD3 may be used for:

1. Enumeration of total T lymphocytes in bone marrow, blood and other body fluids.
2. Identification and localization of normal and malignant T lymphocytes, lymphoid and other tissues.
3. Identification of leukemias and lymphomas of T cell origin.
4. T lymphocyte activation studies.

Assayed by flow cytometric analysis using 10 µl of the conjugate to stain 1 x 10^6 cells. Maximum signal to background and maximum percent positive are obtained.

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

Direct Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied

1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A,or heparin anticoagulant **OR**
   b. Human cell suspension (e.g., peripheral blood mononuclear cells isolated on HISTOPAQUE®, Catalog Number 10771

2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. Quantum Red conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Catalog No. R2138).
4. 12 x 75 mm test tubes.
5. Adjustable micropipette.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue, Catalog No. 302643, 0.2% in 0.01 M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Whole blood lysing solution.
11. Flow cytometer.

Procedure

1. a. Use 100 µl of whole blood **OR**
   b. Adjust cell suspension to 1 x 10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (trypan blue). For each sample, add 100 µl or 1 x 10^6 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 to 22 °C) for 30 minutes. Proper controls to be included for each sample are:
   a. An autofluorescence control: 10 µl Diluent in place of monoclonal antibody, followed by steps 3 - 7.
   b. A negative staining control: 10 µl of Quantum Red conjugated, isotype-matched non-specific mouse immunoglobulin (Product No. R 2138) at the same concentration as test antibody followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of Diluent to all tubes.
5. Pellet cells by centrifugation at 500 x *g* for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. Analyze in a flow cytometer according to manufacturer's instructions.

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.

Storage/Stability

Store at 2-8 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

Beverley, P. C. L., and R. E. Callard, Eur. J. Immunol. **11**, 329 (1981).
Callard, R. E., et al., Clin. Exp. Immunol., **43**, 497 (1981).
Garson, J. A., et al., Nature, **298**, 375 (1982).
Burns, G. F., et al., J. Immunol., **129**, 1451 (1982).
Leucocyte Typing IV, Knapp, W., et al., (eds.), Oxford Press, New York, 1075 (1989).
Reinherz, E.L., et al., Cell, **30**, 715 (1982).
Borst, J., et al., J. Biol. Chem., **258**, 5135 (1983).
Meuer, S., et al., Nature, **303**, 808 (1983).
van dan Elsen, P., et al., Nature, **312**, (1984).
Furley, A.J., et al., Cell, **46**, 75 (1986).
Gold, D.P., et al., Nature, **321**, 4 (1986).
Clevers, H., et al., Annu. Rev. Immunol., **6**, 629 (1988).
Glazer, N.A. and L.J. Stryer, J. Biophys. Soc., **43**, 383 (1982).

AH,PHC 10/10-1