DakoCytomation

Monoclonal Mouse Anti-Human CD3/CY, Clone UCHT1 Code CA696
Monoclonal Mouse Anti-Human CD3/PB, Clone UCHT1 Code PB982

For research use only. Not for use in diagnostic procedures.

Recommended use

CA696 and PB982 are recommended for use in flow cytometry for identification of cells expressing CD3.

Introduction

Human TCR/CD3 is a complex structure on the lymphocyte surface. It consists of the TCRαβ or TCRγδ heterodimer and the associated CD3 complex. The CD3 complex is composed of six polypeptides with usually four different transmembrane CD3 chains, γ (gamma), δ (delta), ε (epsilon), and ζ (zeta). Three different dimers, γε, δε, and ζζ, constitute the CD3 complex. The Mr of CD3ε is 20 000 (1).

The CD3 complex is crucial in transducing antigen-recognition signals into the cytoplasm of T cells and in regulating the cell surface expression of the TCR complex. Further it plays an important role in the differentiation of thymocytes (1).

CD3 is first detectable in early thymocytes and its appearance probably represents one of the earliest signs of commitment to the T cell lineage. In cortical thymocytes, during early stages of maturation, the CD3 antigen is predominantly present in the cell cytoplasm. In medullary thymocytes, the CD3 antigen is predominantly detected on the cell surface (2, 3).

Reagent provided

The Anti-CD3 conjugates, CA696 and PB982, have been produced from a purified monoclonal mouse antibody. Cascade Yellow* (CY) has an excitation and emission spectrum at 406 nm and 541 nm, respectively. Pacific Blue* (PB) has an excitation and emission spectrum at 406 nm and 456 nm, respectively. The conjugates are provided in liquid form in buffer containing 15 mmol/L NaN_3 and 1% bovine serum albumin, pH 7.2. Each vial contains 100 tests (10 µL of conjugate for 100 µL peripheral blood).

Clone: UCHT1. Isotype: IgG1, kappa. Conjugate concentration mg/L: See label on vial.

	Fluorochrome	Control Reagent Code
CA696	Cascade Yellow (CY)	X7908
PB982	Pacific Blue (PB)	X0987

* The Cascade Yellow™ and Pacific Blue™ dye antibody conjugates in these products are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with microarrays, and are covered by pending and issued patents.

Immunogen

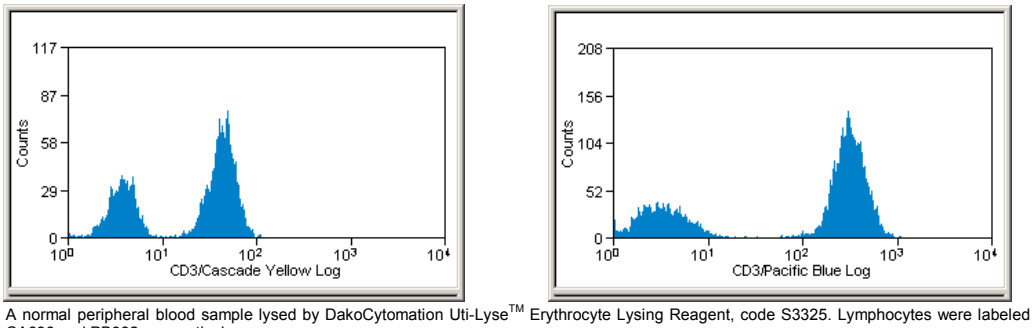
Human infant thymocytes and lymphocytes from a patient with Sézary disease (4).

Specificity

Anti-CD3, UCHT1, was included in the First and Third International Workshops and Conferences on Human Leucocyte Differentiation Antigens, and studies by a number of laboratories confirmed its reactivity with the CD3 antigen (5). Anti-CD3, UCHT1, reacts with the 20 kDa ε-chain of CD3 (6).

Anti-CD3 labels T cells in thymus, spleen, tonsil and blood (2-4). It also labels Purkinje cells in the cerebellum, which is the only other cell type known to bind antibodies to CD3 (7).

The antibody is able to induce in vitro proliferation of mature thymocytes and T cells in the presence of interleukin-2 (IL-2) (8).



A normal peripheral blood sample lysed by DakoCytomation Uti-Lyse™ Erythrocyte Lysing Reagent, code S3325. Lymphocytes were labeled with CA696 and PB982, respectively.

Precautions

1. The device is not intended for clinical use including diagnosis, prognosis, and monitoring of a disease state, and it must not be used in conjunction with patient records or treatment.

2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

3. As with any product derived from biological sources, proper handling procedures should be used.

Storage

Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services.

Staining procedure

1. Transfer 100 µL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube.
2. Add 10 µL of fluorochrome-conjugated Anti-CD3 and mix gently by using a vortex mixer.

DakoCytomation Denmark A/S · Produktionsvej 42 · DK-2600 Glostrup · Denmark · Tel. +45 44 85 95 00 · Fax +45 44 85 95 95 · CVR No. 33 21 13 17

3. Incubate in the dark at 2-8 °C for 30 minutes or at room temperature (20-25 °C) for 15-30 minutes.
4. Add 100 µL of DakoCytomation Uti-Lyse™ (code S 3325) Reagent A to the tube and mix gently by using a vortex mixer. Incubate for 10 minutes at room temperature in the dark.
5. Add 1 mL of DakoCytomation Uti-Lyse™ Reagent B to the tube and mix gently by using a vortex mixer. Incubate for 10 minutes at room temperature in the dark.
6. Centrifuge at 300 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid in the tube.
7. Add 2 mL of PBS (DakoCytomation code S3024) to the tube and resuspend the cells by using a vortex mixer.
8. Repeat step 6.
9. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS.
10. Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples should be analysed within 24 hours after staining.

Note that fluorochrome conjugates are light sensitive, and samples should be protected from light during the staining procedure and until the analysis.

Procedural notes

Step 1: It is optional to include a suitable positive and negative control sample with each run for reagent and preparation control.

Step 2: The volume of conjugate recommended is a guideline only. Optimal staining conditions may vary depending on specimen and preparation method and should be determined by each individual laboratory.

It is optional to include a control reagent test tube. The control reagent should match the conjugated antibody reagent. Recommended control reagents are shown in the table above.

Steps 4 and 5: If another cell-lysing reagent is used, please follow the recommendations for that reagent. Note that if the alternative lysing reagent does not contain fixative, e.g. DakoCytomation EasyLyse™, code S2364, the PBS in step 9 should contain 1% paraformaldehyde unless the sample is analysed within the time frame recommended for the lysing reagent.

Multicolour reagents are preferable to single-colour reagents for the comprehensive analysis of flow cytometry specimens. The correct use of colour compensation is particularly important in multicolour analysis.

Statement of quality

Each lot of reagent is tested by flow cytometry for conformance with characteristics of a standard reagent. In this quality control test, 10 µL CA696 and PB982 are used for 100 µL cell suspension containing up to 10^6 leucocytes from normal human peripheral blood. The control reagents are X7908 and X0987, respectively.

References

1. Saito T, Yamazaki T. TC4. CD3 workshop panel report. In: Kishimoto T, Kikutani H, von dem Borne AEG, Goyert SM, Mason DY, Miyasaka M, et al., editors. Leucocyte typing VI. White cell differentiation antigens. Proceedings of the 6th International Workshop and Conference; 1996 Nov 10-14; Kobe, Japan. New York, London: Garland Publishing Inc.; 1997. p. 44-48.
2. Campana D, Thompson JS, Amlot P, Brown S, Janossy G. The cytoplasmic expression of CD3 antigens in normal and malignant cells of the T lymphoid lineage. J Immunol 1987;138:648-55.
3. Swerdlow SH, Angermeier PA, Hartman AL. Intrathymic ontogeny of the T cell receptor associated CD3 (T3) antigen. Lab Invest 1988;58:421-7.
4. Beverley PC, Callard RE. Distinctive functional characteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody. Eur J Immunol 1981;11:329-34.
5. McMichael AJ, Gotch FM. T-cell antigens: new and previously defined clusters. In: McMichael AJ, Beverley PCL, Cobbold S, Crumpton MJ, Gilks W, Gotch FM, et al., editors. Leucocyte typing III. White cell differentiation antigens. Proceedings of the 3rd International Workshop and Conference; 1986 Sept 21-26; Oxford, England. Oxford, New York, Tokyo: Oxford University Press; 1987. p.31-62.
6. Tunnacliffe A, Olsson C, Traunecker A, Krissansen GW, Karjalainen K, de la Hera A. T3.2. The majority of CD3 epitopes are conferred by the epsilon chain. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leucocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 295-6.
7. Garson JA, Beverley PC, Coakham HB, Harper EI. Monoclonal antibodies against human T lymphocytes label Purkinje neurones of many species. Nature 1982;298:375-7.
8. Denning SM, Tuck DT, Singer KH, Haynes BF. T2.11. Activation of human thymocytes via CD3 and CD2 molecules. In: McMichael AJ, Beverley PCL, Cobbold S, Crumpton MJ, Gilks W, Gotch FM et al, editor. Leukocyte Typing III. White cell differentiation antigens. Proceedings of the 3rd International Workshop and Conference. Oxford-New York-Tokyo: Oxford University Press; 1987. p. 144-7.

Explanation of symbols

REF	Catalogue number		Keep away from sunlight (consult storage section)		Manufacturer
	Consult instructions for use	LOT	Batch code		
2 °C 8 °C	Temperature limitation		Use by		

DakoCytomation Denmark A/S · Produktionsvej 42 · DK-2600 Glostrup · Denmark · Tel. +45 44 85 95 00 · Fax +45 44 85 95 95 · CVR No. 33 21 13 17