

Monoclonal Mouse Anti-Human CD45, Leucocyte Common Antigen/CY, Clone T29/33
Monoclonal Mouse Anti-Human CD45, Leucocyte Common Antigen/PB, Clone T29/33

Code CA697
Code PB986

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

Recommended use CA697 and PB986 are recommended for use in flow cytometry for identification of cells expressing CD45.

Introduction CD45 is a single-chain type I transmembrane protein typically expressed at high levels on all nucleated cells of haematopoietic origin (1). Thus, CD45 is present on T and B-lymphocytes, granulocytes, monocytes and macrophages, with the exception of maturing erythrocytes and megakaryocytes (2). There are five different isoforms of CD45, based on differential splicing of exons 4, 5 and 6, named ABC, AB, BC, B and 0. The Mr of the isoforms ranges from 220 000 for the ABC isoform to 180 000 for the 0 isoform. All the CD45 isoforms share the same intracellular segment which has been shown to have tyrosine phosphatase activity and which has a functional role in lymphocyte activation and differentiation (1).

Antibodies that recognize all five isoforms are known as anti-CD45 (1).

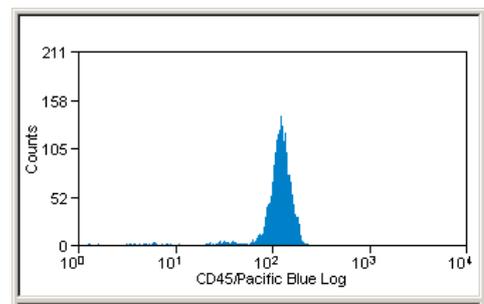
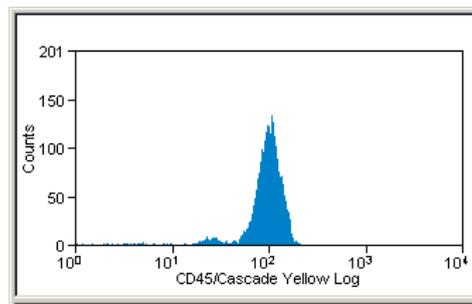
Reagent provided The Anti-CD45 conjugates CA697 and PB986 have been produced from a purified monoclonal mouse antibody. Cascade Yellow* (CY) has an excitation and emission spectrum at 406 nm and 541 nm, respectively. Pacific Blue* (PB) has an excitation and emission spectrum at 406 nm and 456 nm, respectively. The conjugates are provided in liquid form in buffer containing 15 mmol/L NaN₃ and 1% bovine serum albumin, pH 7.2. Each vial contains 100 tests (10 µL of conjugate for 100 µL peripheral blood).

Clone: T29/33. Isotype: IgG1, kappa. Conjugate concentration mg/L: See label on vial.

Antibody Code	Fluorochrome	Control Reagent Code
CA697	Cascade Yellow (CY)	X7908
PB986	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.

Specificity Anti-CD45, T29/33, was included in the Third and Fourth International Workshops and Conferences on Human Leucocyte Differentiation Antigens. At the Third Workshop, the antibody was clustered as a CD45 antibody reacting with **all** the known isotypes of the CD45 family, also called the leucocyte common antigen family (3). At the Fourth Workshop, the expression of CD45 in a range of haematopoietic cell lines, and the lack of CD45 in non-haematopoietic cell lines, were demonstrated. Notably, CD45 was expressed at a very low level in the U-266 plasmacytoid cell line (4).



A normal peripheral blood sample lysed by DakoCytomation Uti-Lyse™ Erythrocyte Lysing Reagent, code S3325. Lymphocytes were labeled with CA697 and PB986, 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 (NaN₃), 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-CD45 and mix gently by using a vortex mixer.
 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 CA697 and PB986 are used for 100 µL cell suspension containing up to 10⁶ leucocytes from normal human peripheral blood. The control reagents are X7908 and X0987, respectively.

References

1. Sewell WA, Cooley MA, Hegen M. NL6. CD45 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. 499-502.
2. Sewell WA, Cooley MA, Hegen M. NL6. CD45 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. 499-502.
3. Cobbold S, Hale G, Waldmann H. Non-lineage, LFA-1, and leucocyte common antigens: new and previously defined clusters. In: McMichael AJ, Beverley PCL, Cobbold S, Crumpton MJ, Gilks W, Gotch FM, et al., editors. Leukocyte typing III. White cell differentiation antigens. Proceedings of the 3rd International Workshop and Conference; 1986 Sep 21-26; Oxford, England. Oxford, New York, Tokyo: Oxford University Press; 1987. p. 788-803..
4. Schwinzer R. N7. Cluster report: CD45/CD45R. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leukocyte 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. 628-34.

Explanation of symbols

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