Monoclonal Mouse Anti-Human CD8/PB

Clone DK25

Code No. PB 984

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

Recommended use

Monoclonal Mouse Anti-Human CD8/PB, is recommended for use in flow cytometry for identification of cells expressing CD8.

Introduction

CD8 is a 68 kDa disulphide-linked, transmembrane glycoprotein. The CD8 molecule serves as a receptor for MHC class I molecules and can mediate a function as a coreceptor in TCR-ligand binding and T-cell activation. Its cytoplasmic part is associated with the $p56^{lck}$ tyrosine kinase (1).

CD8 is expressed by the great majority of cortical thymocytes and approximately 30% of medullary thymocytes, and by class I major histocompatibility complex restricted, mature suppressor/cytotoxic T cells. In addition a proportion of $\gamma\delta$ T cells and NK cells express CD8 (1). Thus, CD8 is expressed by about 25-35% of peripheral T cells, and about 30% of NK cells express low levels of CD8 (2).

Reagent provided

PB 984 is a purified monoclonal mouse antibody conjugated with Pacific Blue* (PB). PB has an excitation and emission spectrum at 406 nm and 456 nm, respectively. The conjugate is provided in liquid form in 0.05 mol/L Tris/HCl, 15 mmol/L NaN_3, pH 7.2, 1% bovine serum albumin. Each vial contains 100 tests (10 µL of conjugate for 100 µL peripheral blood).

Clone: DK25. Isotype: IgG1, kappa. Conjugate concentration mg/L: See label on vial.

* The Pacific Blue™ dye antibody conjugate in this product is sold under license from Molecular Probes, Inc., for research use only, except for use in combination with microarrays, and is covered by pending and issued patents.

Specificity

The specificity of Anti-CD8, DK25, is equivalent to that of the CD8-clustered antibodies Leu-2a.

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 PB 985 and mix gently with a vortex mixer. The 10 µL is a guideline only; the optimal volume should be determined by the individual laboratory.
3. The recommended negative control is a non-reactive PB-conjugated antibody of the same isotype.
4. Incubate in the dark at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15-30 minutes.
5. Add 100 µL of Dako Uti-Lyse™ (code Nos. S 3325 or S 3350) Reagent A to each sample and mix gently with a vortex mixer. Incubate for 10 minutes at room temperature in the dark.
6. Add 1 mL of Dako Uti-Lyse™ Reagent B to each sample and mix gently with a vortex mixer. Incubate for 10 minutes at room temperature in the dark. If another lysing reagent is used in steps 5 and 6, please follow the recommendations for that reagent.
7. Centrifuge at 300 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
8. Add 2 mL 0.01 mol/L PBS containing 2% bovine serum albumin and resuspend the cells by using a vortex mixer.
9. Repeat step 7.
10. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS. The PBS should contain 1% paraformaldehyde (fixative) if samples are not analysed the same day.
11. Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. It is recommended to include a suitable positive and negative control sample with each run for reagent and preparation control. Note that fluorochrome conjugates are light sensitive, and samples should be protected from light during the staining procedure and until the analysis.

References

1. Nakauchi H. TC9. CD8 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. 65-7.
2. Leong AS-Y, Cooper K, Leong FJW-M. Manual of diagnostic antibodies for immunohistology. 2nd Edition. London: Greenwich Medical Media Ltd.; 2003. p. 73-74.

Explanation of symbols

REF	Catalogue number		Keep away from sunlight (consult storage section)		Manufacturer
	Consult instructions for use	LOT	Batch code	EC REP	Authorized representative in the European Community
2 °C 8 °C	Temperature limitation		Use by		

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