Monoclonal Mouse Anti-Human CD28/RPE

Clone CD28.1

Code No. R 7164

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

Recommended use

Monoclonal Mouse Anti-Human CD28/RPE, is recommended for use in flow cytometry for identification of cells expressing CD28.

Introduction

CD28 was initially termed T44 or Tp44 (1,2), but was designated CD28 at the Third International Workshop and Conference on Human Leucocyte Differentiation Antigens (3). CD28 is a type I transmembrane protein belonging to the Ig superfamily (2, 4) and is a 90 kDa disulphide-linked homodimer with a subunit molecular mass of 44 kDa (4, 5). CD28 is expressed on approximately 95% of $CD4^+$ and 50% of $CD8^+$ T cells, respectively (5). CD28 mediates cell adhesion through the two ligands, CD80 (B7-1) and CD86 (B7-2) (6), expressed on activated B cells. Cross-blocking of CD28 induces T cell activation (7), suggesting an important role for CD28 in the interaction between B and T cells. The CD28 homologue CD152 (CTLA-4), shares the same ligands. However, this interaction inhibits T cell activation (8).

Reagent provided

Purified monoclonal mouse antibody conjugated with R-phycoerythrin (RPE). The conjugate is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) and 15 mmol/L NaN_3, pH 7.2. Each vial contains 100 tests (10 µL of conjugate for up to 10^6 leucocytes from normal human peripheral blood).

Clone: CD28.1 (9). Isotype: IgG1, kappa. Conjugate concentration mg/L: See label on vial.

Immunogen

DCD28.1.3.3. murine T cell hybridoma transfected with human CD28 cDNA (9).

Specificity

Anti-CD28, CD28.1, was included in the Fifth International Workshop and Conference on Human Leucocyte Differentiation Antigens (7) and a number laboratories have confirmed the reactivity against the CD28 antigen. The antibody reacts with a majority of T-cells in human peripheral blood (PBL).

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 R 7164 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 RPE-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. Moretta A, Pantaleo G, Lopez Botet M, Moretta L. Involvement of T44 molecules in an antigen-independent pathway of T cell activation. Analysis of the correlations to the T cell antigen-receptor complex. J Exp Med 1985;162:823-38.
2. Ledbetter JA, Martin PJ, Spooner CE, Wofsy D, Tsu TT, Beatty PG, et al. Antibodies to Tp67 and Tp44 augment and sustain proliferative responses of activated T cells. J Immunol 1985;135:2331-6.

3. McMichael AJ, Gotch FM. 5.1 T-cell antigens: new and previously defined clusters. In: Stockinger H, Majdic O, Köller U, Knapp W. B1.17. Subclustering of the CD24 cluster. 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. 31-65.
4. Arrufo A, Seed B. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. Proc Natl Acad Sci (USA) 1987;84:8573-7.
5. June CH, Ledbetter JA, Linsley PS, Thompson CB. Role of the CD28 receptor in T-cell activation. Immunol Today 1990;11:211-6.
6. Freeman GJ, Gribben JG, Boussiotis VA, Ng JW, Restivo VA Jr, Lombard LA, et al. Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation. Science 1993;262:909-11.
7. Olive D, Cerdan C, Costello R, Sielleur I, Ragueneau, Pages F, Klasen S, Nunes J, Imbert J. CD28 and CTLA-4 cluster report. In: Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, et al., editors. Leukocyte typing V. White cell differentiation antigens. Proceedings of the 5th International Workshop and Conference; 1993 Nov 3-7; Boston, USA. Oxford, New York, Tokyo: Oxford University Press; 1995. p. 360-70.
8. Linsley PS, Wallace PM, Johnson J, Gibson MG, Greene JL, Ledbetter JA, et al. Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. Science 1992;257:792-5.
9. Nunès J, Klasen S, Raguneau M, Pavon C, Couez D, Mawas C, Bagnasco M, Olive D. CD28 mAbs with distinct binding properties differ in their ability to induce T cell activation: analysis of early and late activation events. Int Immunol 1993;5:311-5.

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		

Agilent Technologies Singapore (International) Pte Ltd.
No. 1 Yishun Avenue 7
Singapore, 768923
Tel. +44 161 492 7050
www.agilent.com

Revision 2020.11