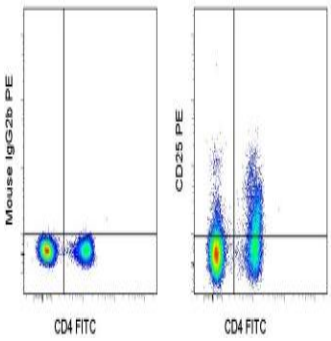
CD25 PE

Also Known As: Interleukin-2 Receptor alpha, IL-2Ra

Catalog Number(s): 9012-0257-025 (25 tests), 9012-0257-120 (120 tests)

CE IVD



Staining of normal human peripheral blood lymphocytes with CD4 FITC and Mouse IgG2b PE (left) or CD25 PE (right).

Product Information

Contents: CD25 PE

REF Σ **Catalog Number(s):** 9012-0257-025 (25 tests), 9012-0257-120 (120 tests)

Clone: CD25-4E3

Concentration: 5 uL (0.125 ug)/test (a test is defined as the amount that will stain 1 x 10e6 cells in 100 uL)

Host/Isotype: Mouse IgG2b

Formulation: Aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer.

Storage Conditions: Store at 2-8°C. Do not freeze.

Light-sensitive material.

Caution, contains Azide

Manufacturer: eBioscience, Inc., 10255 Science Center Drive, San Diego, CA 92121, USA

EC REP **Authorized Representative:** Bender MedSystems GmbH, an eBioscience Company Campus Vienna Biocenter 2 A-1030 Vienna Austria

Intended Use

The CD25-4E3 fluorochrome-conjugated monoclonal antibody reacts with the human CD25 antigen. CD25 can be detected in human biological samples using immunological techniques.

Principles of the Test

Flow cytometry is a useful tool for simultaneously measuring multiple physical properties of individual particles (such as cells). Cells pass single-file through a laser beam. As each cell passes through the laser beam, the cytometer records how the cell or particle scatters incident laser light and emits fluorescence. Using this flow cytometric analysis protocol, one can perform a simultaneous analysis of surface molecules at the single-cell level.

Description

This CD25-4E3 monoclonal antibody reacts to human CD25, which is also known as the low-affinity interleukin (IL)-2 receptor alpha. CD25 is expressed on a subet of T cells (many of which are regulatory T cells), activated T and B cells, and macrophages. This receptor subunit associates with CD122, the IL-2 receptor beta chain, and CD132, the common gamma chain, to form the high-affinity IL-2 receptor. IL-2 receptor signaling has been linked to cell proliferation and survival in lymphocytes.

Specimen Collection and Storage Instructions

Collect venous blood sample by venipuncture into a sterile blood collection tube using an appropriate anticoagulant (EDTA is recommended). Keep samples at room temperature (18-25°C). Prior to use, mix samples by gentle agitation.

Materials Required But Not Provided

- 12x75 mm test tubes
- Buffers (eBioscience Flow Cytometry Staining Buffer, Cat. No. 00-4222 recommended)
- Lysis Buffer (eBioscience 1X RBC Lysis Buffer, Cat. No. 00-4333 or eBioscience 1-step Fix/Lyse Solution (10X), Cat. No. 00-5333 recommended)
- For intracellular staining use IC Fixation Buffer and Permeabilization Buffer, Cat. No. 88-8823 (intracellular cytokine or cytoplasmic protein staining) or Foxp3 Buffer Set, Cat. No. 00-5523 (For nuclear protein staining). Refer to the Best Protocols section of the eBioscience website for the "Staining Intracellular Antigens for Flow Cytometry" protocols.
- Viability stain (7-AAD Viability Staining Solution, Cat. No. 00-6993 or Propidium Iodide Staining Solution, Cat. No. 00-6990 recommended)
- Automated pipettes
- Centrifuge
- Vortex mixer
- Ice bucket or refrigerator
- Flow cytometer

Test Protocol

NOTE: For intracellular staining, refer to the Best Protocols section of the eBioscience website for the "Staining Intracellular Antigens for Flow Cytometry" protocols.

1. Aliquot 100 µL of the test sample into tubes.
2. Add 5 µL of the appropriate antibody to each tube.
3. Incubate 30-60 minutes at 2-8°C. Alternatively, samples can be incubated at room temperature in the dark 15-30 minutes.
4. Add 2 ml of 1X RBC Lysis Buffer (at room temperature) per tube. Mix gently. (Alternatively, samples can be incubated with 2 mL 1-step Fix/Lyse Solution.)
5. Incubate samples in the dark at room temperature for 10 minutes. Do not exceed 15 minutes of incubation with the RBC Lysis Buffer.
6. Centrifuge samples at 300-400 x g for 5 minutes at room temperature, decant/aspirate supernatant and wash 1 time with 2 ml of Flow Cytometry Staining Buffer.
7. Centrifuge samples at 300-400 x g for 5 minutes at room temperature, decant/aspirate supernatant.
8. Resuspend stained cell pellet in 1 mL Flow Cytometry Staining Buffer and analyze samples on a flow cytometer.

Limitations

1. For optimal performance of fluorochrome conjugated antibodies, store vials at 2-8°C in the dark. Do not freeze.
2. Centrifuge the antibody vial prior to opening to recover the maximum volume.
3. Except where noted in the protocol, all staining should be done on ice or at 2-8°C with minimal exposure to light.

Performance Characteristics

Consistency of high-quality reagents is ensured by testing each lot of monoclonal antibody for conformance against characteristics of a standard reagent. Representative flow cytometric data is included where appropriate.

Evidence of Deterioration

For questions or concerns regarding the performance or quality of products received, please contact eBioscience Technical Support (see below).

References

Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (H3-A6), 3rd Edition published by the National Committee for Clinical Laboratory Standards.

Jackson AL, Matsumoto H, Janszen M, Maino V, Blidy A, Shye S. Restricted expression of p55 interleukin 2 receptor (CD25) on normal T cells. Clin Immunol Immunopath. 1990;54:126-133

Ng WF, Duggan PJ, Ponchel F, et al. Human CD4+CD25+ cells: a naturally occurring population of regulatory T cells. Blood. 2001;98:2736-2744.