



CD25 antibodies mouse

CD25-PE	130-091-013
CD25-APC	130-093-734
CD25-Biotin	130-092-569

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1. Description

Components	1 mL CD25 antibodies, mouse: monoclonal CD25 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	7D4 (isotype: rat IgM).
Capacity	100 tests or up to 10^9 total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

CD25, the low affinity interleukin-2 receptor α chain (IL-2R α), is expressed in the early phase (CD4⁺CD8⁻) of thymic T cell development, as well as on activated T and B cells, and at a lower level on activated monocytes. CD25 forms the high affinity IL-2 receptor complex together with the β chain (CD122) and γ chain (CD132). A subpopulation of CD4⁺CD25⁺ T cells is supposed to act as regulatory T cells upon activation.¹ Regulatory CD4⁺CD25⁺ T cells appear to suppress harmful immunological reactions to self or foreign antigens. In transfer experiments it has been shown that CD4⁺CD25⁺ T cells can prevent autoimmune reactions and maintain or reconstitute tolerance.^{2,3} The 7D4 antibody does not inhibit the binding of IL-2.^{4,5}

1.2 Applications

- Identification and enumeration of activated B cells and monocytes, regulatory T cells, or Pro-T cells (double negative thymocytes) in various tissues by fluorescence microscopy or flow cytometry.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse CD25⁺ cells can be isolated by using the CD25 MicroBead Kit (# 130-091-072).

1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

CD25 conjugate	PE	APC	Biotin
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11
Immunohistochemistry^c			

a) Given antibody dilutions are for a cell concentration of up to 10^7 cells/100 μ L of buffer.
b) For optimal results, cells must be stained prior to fixation.
c) The optimal antibody dilution should be determined.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS) pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
▲ Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD25-Biotin.
- (Optional) CD4-FITC (# 130-091-608).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μ L of buffer.

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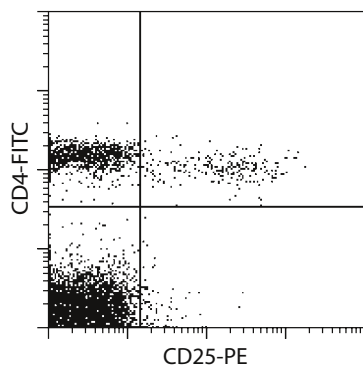
4. Add 10 μ L of the CD25 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at $300\times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD25-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

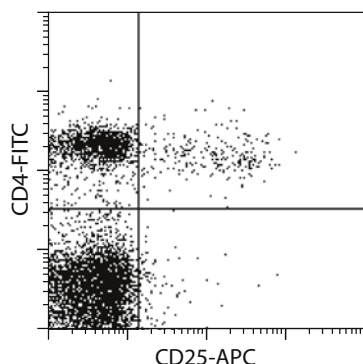
3. Examples of immunofluorescent staining with CD25 antibodies

Mouse spleen cells were stained with CD25-PE (a) or CD25-APC (b) and CD4-FITC and analyzed by flow cytometry. Cells labeled with CD25-Biotin (c) were stained with Anti-Biotin-PE as well as CD4-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

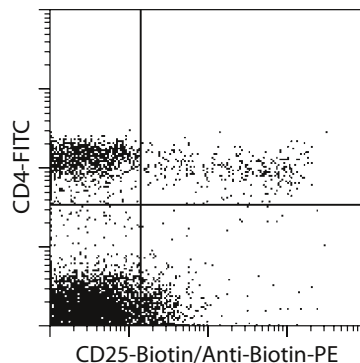
(a) Mouse spleen cells stained with CD25-PE and CD4-FITC.



(b) Mouse spleen cells stained with CD25-APC and CD4-FITC.



(c) Mouse spleen cells stained with CD25-Biotin, Anti-Biotin-PE, and CD4-FITC.



4. References

1. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25). *J. Immunol.* 155: 1151–1164.
2. Shevach EM (2001) Certified professionals: CD4⁺CD25⁺ suppressor T cells. *J. Exp. Med.* 193: F41–F45.
3. Maloy KJ, Powrie F (2001) Regulatory T cells in the control of immune pathology. *Nature Immunol.* 2: 816–822.
4. Ortega RG, Robb RJ, Shevach EM, Malek TR (1984) I. Monoclonal antibodies that define distinct functional epitopes on activated T cells and react with activated B cells. *J. Immunol.* 133: 1970–1975.
5. Malek TR, Robb RJ, Shevach EM (1983) Identification and initial characterization of a rat monoclonal antibody reactive with the murine interleukin 2 receptor-ligand complex. *Immunology* 80: 5694–5698.

All protocols and data sheets are available at www.miltenyibiotec.com.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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