



Miltenyi Biotec

CD134 (OX40) antibodies human

CD134 (OX40)-PE	130-095-270
CD134 (OX40)-APC	130-095-272
CD134 (OX40)-Biotin	130-095-274

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1. Description

Components	1 mL CD134 (OX40) antibodies, human: monoclonal CD134 (OX40) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	ACT35 (isotype: mouse IgG1).
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

CD134 (OX40) is a member of the tumor necrosis factor/nerve growth factor receptor (TNFR/NGFR) family. CD134 is a 50 kDa type I membrane glycoprotein expressed by activated T lymphocytes. The interaction of CD134 with OX40L has been implicated in T cell-dependent humoral response, regulation of primary T cell expansion, survival of T cells, size of the memory T cell pool, and regulation of tolerance in the CD4⁺ T cell compartment.¹

1.2 Applications

- Identification and enumeration of CD134 (OX40)⁺ cells by flow cytometry or fluorescence microscopy.
- Detection of antigen-specific CD4⁺ T cells.²

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD134 (OX40) 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) The indicated antibody dilutions are for up to 10⁷ cells/100 µL of buffer.
 b) For optimal results, cells must be stained prior to fixation.
 c) The optimal antibody dilution should be determined by the user.

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 human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-VioBlue® (# 130-094-669), 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 CD134 (OX40)-Biotin.
- (Optional) Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD4 (VIT4)-VioBlue (# 130-094-153), CD4 (VIT4)- FITC (# 130-092-358), CD8-VioBlue (# 130-094-152), CD25-PE (# 130-091-024), or CD25-APC (# 130-092-858). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Mouse IgG1-PE (# 130-092-212), Mouse IgG1-APC (# 130-092-214), or Mouse IgG1-Biotin (# 130-093-018) for isotype control.
- (Optional) CytoStim, human (# 130-092-172, # 130-092-173) for rapid and efficient stimulation of human CD4⁺ and CD8⁺ T cells.
- (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.

140-002-8931.01

2. General protocol for immunofluorescent staining

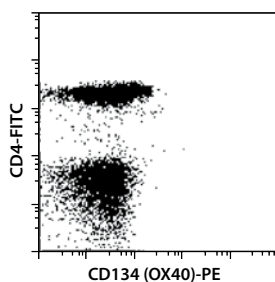
▲ Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated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

1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the CD134 (OX40) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD134 (OX40)-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-VioBlue, 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.

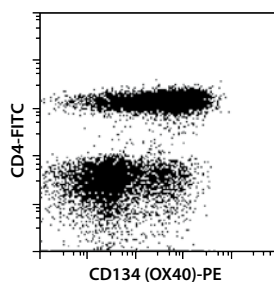
3. Examples of immunofluorescent staining with CD134 (OX40) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stimulated with CytoStim (# 130-092-172, 1:50, 14 h) or left untreated and stained with CD134 (OX40) antibodies conjugated to PE (A), or APC (B) as well as with CD4 (VIT4)-FITC (# 130-092-358). Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD134 (OX40)-Biotin (C) were stained with Anti-Biotin-APC (# 130-090-856). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

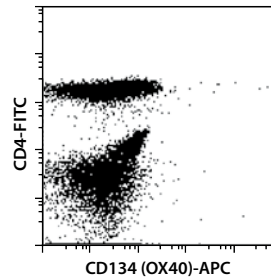
A) Unstimulated cells



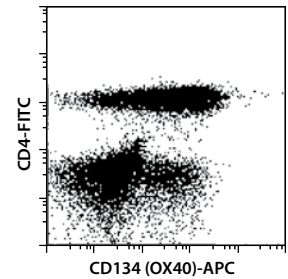
Stimulated cells



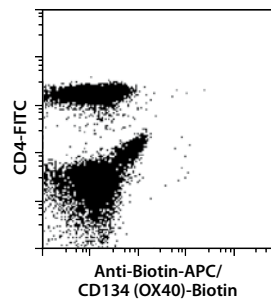
B) Unstimulated cells



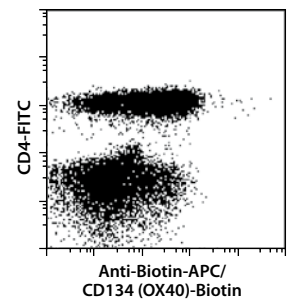
Stimulated cells



C) Unstimulated cells



Stimulated cells



4. References

1. Croft, M *et al.* (2009) The significance of OX40 and OX40L to T-cell biology and immune disease. *Immunol. Rev.* 229: 173–191.
2. Zaunders *et al.* (2009) High levels of human antigen-specific CD4⁺ T cells in peripheral blood revealed by stimulated coexpression of CD25 and CD134 (OX40). *J. Immunol.* 183: 2827–2836.

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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