# CD134 (OX40) antibodies, human

## For research use only

One test corresponds to labeling of up to $10^7$ cells in a total volume of 100 µL.

| Product | Content | Order no. |
|---|---|---|
| CD134 (OX40)-VioBright FITC | for 30 tests | 130-109-664 |
| CD134 (OX40)-VioBright FITC | for 100 tests | 130-109-605 |
| CD134 (OX40)-PE | for 30 tests | 130-109-660 |
| CD134 (OX40)-PE | for 100 tests | 130-109-601 |
| CD134 (OX40)-APC | for 30 tests | 130-109-661 |
| CD134 (OX40)-APC | for 100 tests | 130-109-602 |
| CD134 (OX40)-PE-Vio770 | for 30 tests | 130-109-662 |
| CD134 (OX40)-PE-Vio770 | for 100 tests | 130-109-603 |
| CD134 (OX40)-APC-Vio770 | for 30 tests | 130-109-663 |
| CD134 (OX40)-APC-Vio770 | for 100 tests | 130-109-604 |
| CD134 (OX40)-Biotin | for 30 tests | 130-109-659 |
| CD134 (OX40)-Biotin | for 100 tests | 130-109-600 |

## 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.

## Technical data and background information

| | |
|---|---|
| **Antigen** | CD134 (OX40) |
| **Clone** | REA621 |
| **Isotype** | recombinant human IgG1 |
| **Isotype control** | REA Control (S) antibodies |
| **Alternative names of antigen** | OX-40, OX40 |
| **Molecular mass of antigen [kDa]** | 27 |
| **Distribution of antigen** | B cells, endothelial cells, fibroblasts, lymphocytes, T cells |
| **Product format** | Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide. |
| **Fixation** | Cells should be stained prior to fixation, if formaldehyde is used as a fixative. |
| **Storage** | Store protected from light at 2–8 °C. Do not freeze. |

Clone REA621 recognizes the CD134 (OX40) antigen, 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. It is reported, that CD134 activates NF-κB through its interaction with adaptor proteins TRAF2 and TRAF5.
Additional information: Clone REA621 displays negligible binding to Fc receptors.

## 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). Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

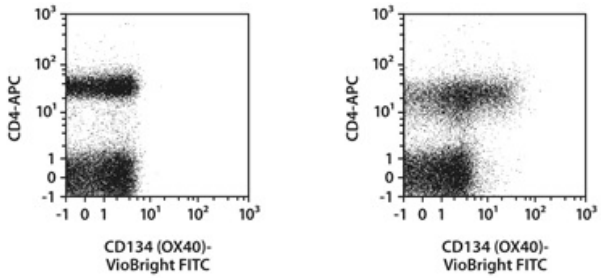
## Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to $10^7$ cells/100 µL of buffer.
- 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 $2\times10^7$ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

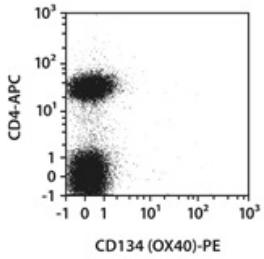
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to $10^7$ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, 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.

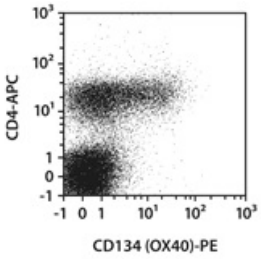
## Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were either left unstimulated (left image) or stimulated with Cytostim at 37 °C for 16 hours. Cells were then stained with CD134 (OX40) antibodies as well as with CD4 antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.


CD4-APC
CD134 (OX40)-
VioBright FITC


CD4-APC
CD134 (OX40)-
VioBright FITC


CD4-APC
CD134 (OX40)-PE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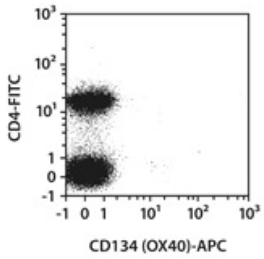
CD134 (OX40)-PE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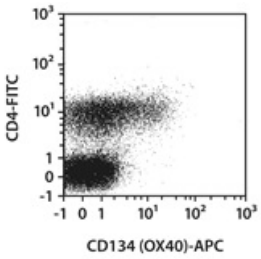
CD134 (OX40)-APC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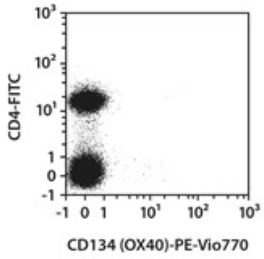
CD134 (OX40)-APC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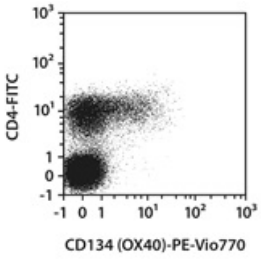
CD134 (OX40)-PE-Vio770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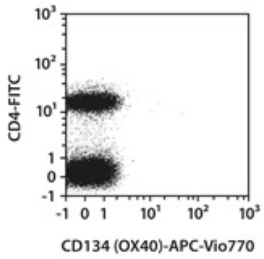
CD134 (OX40)-PE-Vio770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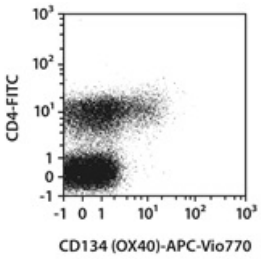
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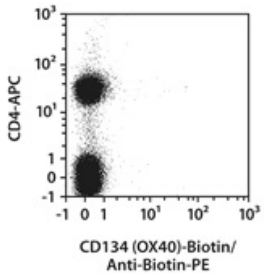
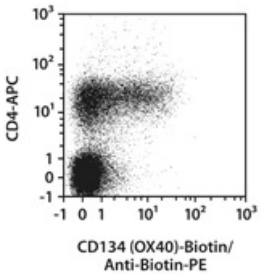
CD134 (OX40)-APC-Vio770


CD4-APC
CD134 (OX40)-Biotin/
Anti-Biotin-PE


CD4-APC
CD134 (OX40)-Biotin/
Anti-Biotin-PE

## References


1. **Kawamata, S. *et al.*** (1998) Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2- and TRAF5-mediated NF-kappaB activation. J. Biol. Chem. 273(10): 5808–5814.
2. **Croft, M. *et al.*** (2009) The significance of OX40 and OX40L to T-cell biology and immune disease. Immunol. Rev. 229(1): 173–191.
3. **Zaunders, J. J. *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.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