CD4 (Domain 1) antibodies, rat
For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of \(10^6\) cells.

<table>
<thead>
<tr>
<th>Product</th>
<th>Content</th>
<th>Order no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 (Domain 1)-FITC</td>
<td>9 µg in 300 µL</td>
<td>130-107-667</td>
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<tr>
<td>CD4 (Domain 1)-FITC</td>
<td>30 µg in 1 mL</td>
<td>130-107-623</td>
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<tr>
<td>CD4 (Domain 1)-PE</td>
<td>9 µg in 300 µL</td>
<td>130-107-668</td>
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<tr>
<td>CD4 (Domain 1)-PE</td>
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<td>130-107-624</td>
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<tr>
<td>CD4 (Domain 1)-APC</td>
<td>9 µg in 300 µL</td>
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<tr>
<td>CD4 (Domain 1)-APC</td>
<td>30 µg in 1 mL</td>
<td>130-107-625</td>
</tr>
<tr>
<td>CD4 (Domain 1)-VioBlue</td>
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<tr>
<td>CD4 (Domain 1)-VioBlue</td>
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<tr>
<td>CD4 (Domain 1)-PE-Vio770</td>
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<td>CD4 (Domain 1)-PE-Vio770</td>
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<tr>
<td>CD4 (Domain 1)-PerCP-Vio700</td>
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<tr>
<td>CD4 (Domain 1)-Biotin</td>
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<tr>
<td>CD4 (Domain 1)-Biotin</td>
<td>30 µg in 1 mL</td>
<td>130-107-621</td>
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</tbody>
</table>

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         CD4
Clone           REA482
Isotype         recombinant human IgG1
Isotype control REA Control antibodies
Alternative names of antigen W3/25, p55, Leu-3
Molecular mass of antigen [kDa] 48
Distribution of antigen dendritic cells, macrophages, monocytes, T cells, thymocytes, T helper 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 REA482 recognizes domain 1 of the rat CD4 antigen, a single-pass type I membrane protein also known as T cell surface antigen T4/Leu-3 or W3/25 antigen. CD4 is a member of the immunoglobulin superfamily and is expressed on the surface of T helper cells, monocytes, macrophages, and dendritic cells. It has four immunoglobulin domains (D1 to D4). D1 and D3 resemble immunoglobulin variable (IgV) domains, and D2 and D4 resemble immunoglobulin constant (IgC) domains. CD4 is a co-receptor that assists the T cell receptor (TCR) in communicating with an antigen-presenting cell (APC). CD4 amplifies the signal generated by the TCR by recruiting the tyrosine kinase Lck, which is essential for activating many molecular components of the signaling cascade of an activated T cell. CD4 also interacts directly with MHC class II molecules on the surface of APCs. Additional information: Clone REA482 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 Ca2+ or Mg2+ 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 1:10 for up to 106 cells/50 µL of buffer.
- Volumes given below are for up to 106 nucleated cells. When working with fewer than 106 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 2×106 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 106 nucleated cells per 45 µL of buffer.
4. Add 5 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**
Splenocytes from Wistar rats were stained with CD4 (Domain 1) antibodies or with the corresponding REA Control antibodies (left image) as well as with CD3 antibodies. Flow cytometry was performed with the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.
References


Warranty

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