

CD3 antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10⁶ cells.

Product	Content	Order no.
CD3-FITC	9 µg in 300 µL	130-109-878
CD3-FITC	30 µg in 1 mL	130-109-836
CD3-PE	9 µg in 300 µL	130-109-879
CD3-PE	30 µg in 1 mL	130-109-837
CD3-APC	9 µg in 300 µL	130-109-880
CD3-APC	30 µg in 1 mL	130-109-838
CD3-PE-Vio770	9 µg in 300 µL	130-109-881
CD3-PE-Vio770	30 µg in 1 mL	130-109-839
CD3-APC-Vio770	9 µg in 300 µL	130-109-882
CD3-APC-Vio770	30 µg in 1 mL	130-109-840
CD3-PerCP-Vio700	9 µg in 300 µL	130-109-883
CD3-PerCP-Vio700	30 µg in 1 mL	130-109-841
CD3-Biotin	9 µg in 300 µL	130-109-877
CD3-Biotin	30 µg in 1 mL	130-109-835

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	CD3
Clone	REA641
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	T cell antigen receptor complex, T3, AI504783, CD3epsilon, T3E, Ctg-3, Ctg3, T3g
Molecular mass of antigen [kDa]	37 (sum of molecular weights of subunits)
Distribution of antigen	NKT cells, T cells, thymocytes
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA641 recognizes the mouse CD3 complex, which is expressed on all mature T lymphocytes, NKT cells, and during the development of thymocytes. CD3, also known as T3, belongs to the Ig superfamily. It consists of CD3 ϵ , δ , γ , and ζ chains and forms a TCR complex by associating with TCR α/β or γ/δ chains. The TCR does not possess intracellular signaling domains, uncoupling Ag recognition from T cell signaling. The TCR is instead non-covalently associated with a multisubunit signaling apparatus, consisting of the CD3 $\epsilon\gamma$ and CD3 $\epsilon\delta$ heterodimers and the CD3 $\zeta\zeta$ homodimer, which collectively form the TCR-CD3 complex. CD3 is involved in T cell activation, signal transduction, and antigen recognition by binding the peptide/MHC antigen complex. Additional information: Clone REA641 displays negligible binding to Fc receptors.

Reagent requirements

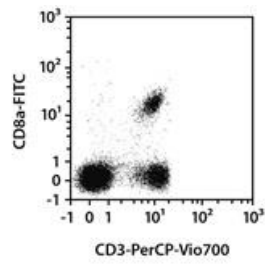
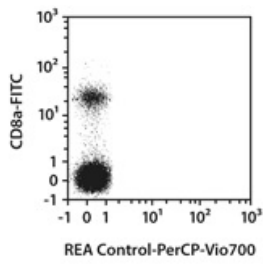
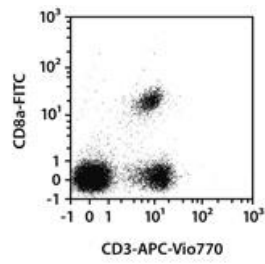
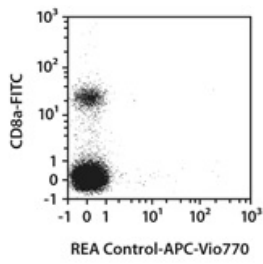
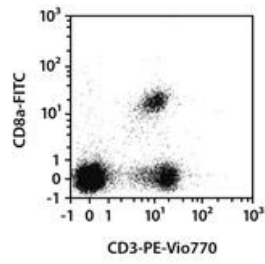
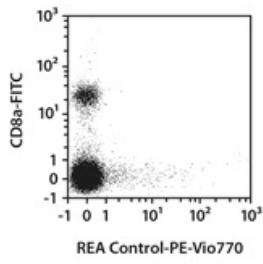
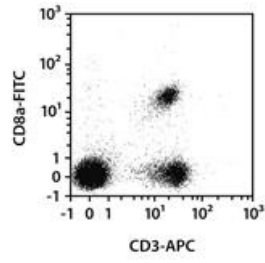
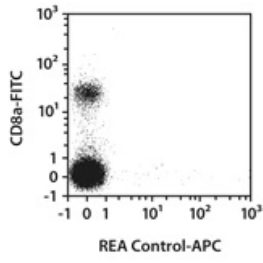
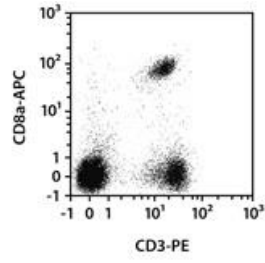
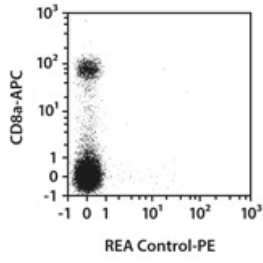
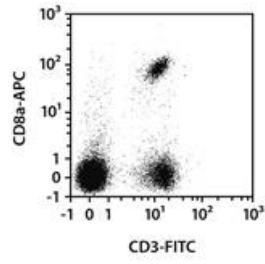
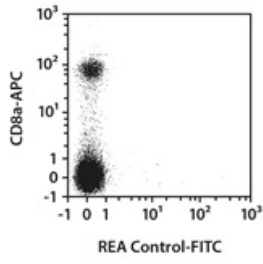
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Note: EDTA can be replaced by other 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.

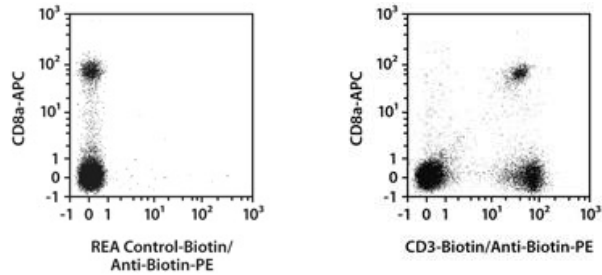
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10⁶ cells/50 μ L 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 \times 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 \times g for 10 minutes. Aspirate supernatant completely.
 3. Resuspend up to 10⁶ nucleated cells per 45 μ L of buffer.
 4. Add 5 μ L 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 \times g for 10 minutes. Aspirate supernatant completely.
 7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L 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 C57BL/6 mice were stained with CD3 antibodies or with the corresponding REA Control antibodies (left images) as well as with CD8a antibodies. Flow cytometry was performed using the MACSQuant[®] Analyzer. 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.





References

1. **Gold, D. P. et al.** (1987) Evolutionary relationship between the T3 chains of the T-cell receptor complex and the immunoglobulin supergene family. *Proc. Natl. Acad. Sci. U.S.A.* 84(21): 7649–7653.
2. **Miescher, G. C. et al.** (1989) Production and characterization of a rat monoclonal antibody against the murine CD3 molecular complex. *Immunol. Lett.* 23(2): 113–118.
3. **Exley, M. et al.** (1991) Structure, assembly and intracellular transport of the T cell receptor for antigen. *Semin. Immunol.* 3(5): 283–297.
4. **Birnbaum, M. E. et al.** (2014) Molecular architecture of the $\alpha\beta$ T cell receptor-CD3 complex. *Proc. Natl. Acad. Sci. U.S.A.* 111(49): 17576–17581.

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