

CD3ε (intracellular) antibodies, human and mouse

For research use only

One test corresponds to labeling of up to 10^6 cells in a total volume of 100 μL

Product	Content	Order no.
CD3ε (intracellular)-Vio515	for 100 tests	130-117-172
CD3ε (intracellular)-FITC	for 30 tests	130-116-288
CD3ε (intracellular)-FITC	for 100 tests	130-116-185
CD3ε (intracellular)-PE	for 30 tests	130-116-289
CD3ε (intracellular)-PE	for 100 tests	130-116-186
CD3ε (intracellular)-APC	for 30 tests	130-116-290
CD3ε (intracellular)-APC	for 100 tests	130-116-187
CD3ε (intracellular)-Vio515	for 30 tests	130-117-263
CD3ε (intracellular)-Vio667	for 30 tests	130-117-264
CD3ε (intracellular)-Vio667	for 100 tests	130-117-173

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	CD3ε (intracellular)
Clone	REA975
Isotype	recombinant human IgG1
Isotype control	REA Control (I) antibodies
Alternative names of antigen	CD3e, CD3epsilon, T3E
Entrez Gene ID	916
Molecular mass of antigen [kDa]	21
Distribution of antigen	T cells, NKT 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 REA975 recognizes an intracellular part of the CD3ε chain (activation epitope). CD3ε is a single-pass type I membrane protein, which is a part of the CD3 complex and a subunit of the TCR complex. The CD3 complex mediates signal transduction in several intracellular pathways and has a role in T cell activation and antigen recognition by binding the peptide/MHC antigen complex. CD3ε is expressed on all mature T lymphocytes, NKT cells, and during the development of thymocytes.

Additional information: Clone REA975 displays negligible binding to Fc receptors.

Reagent requirements

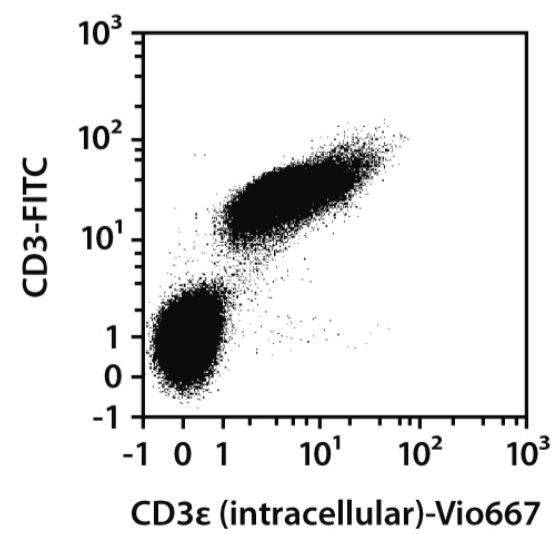
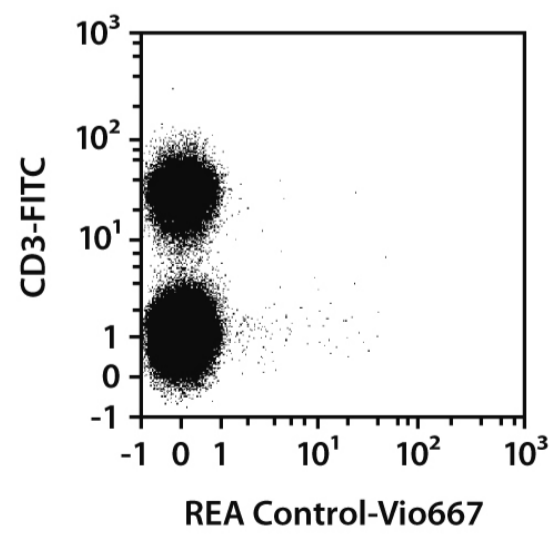
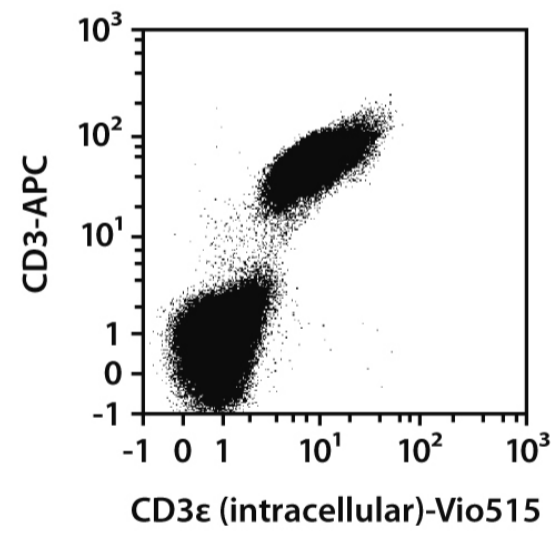
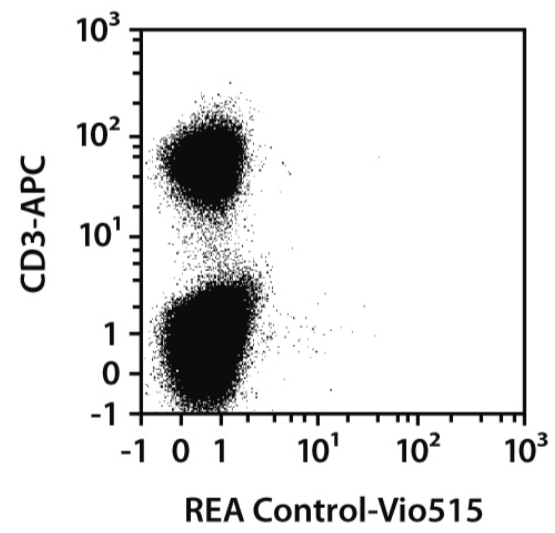
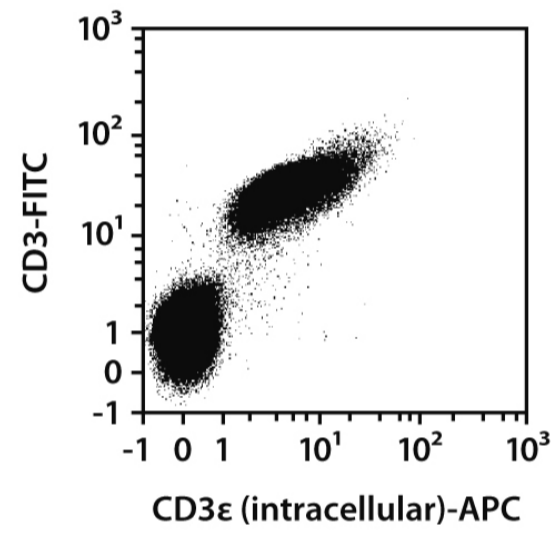
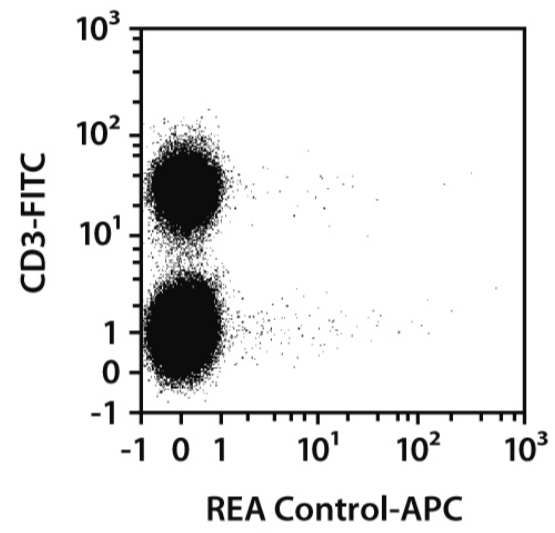
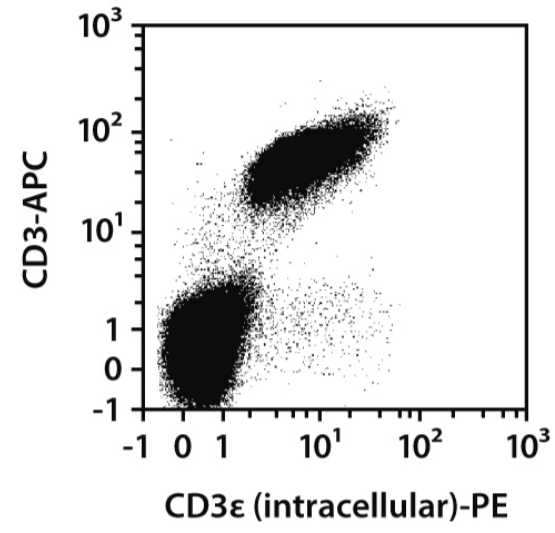
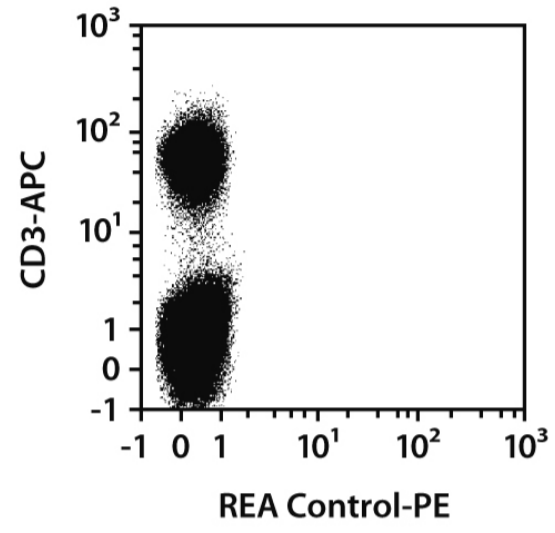
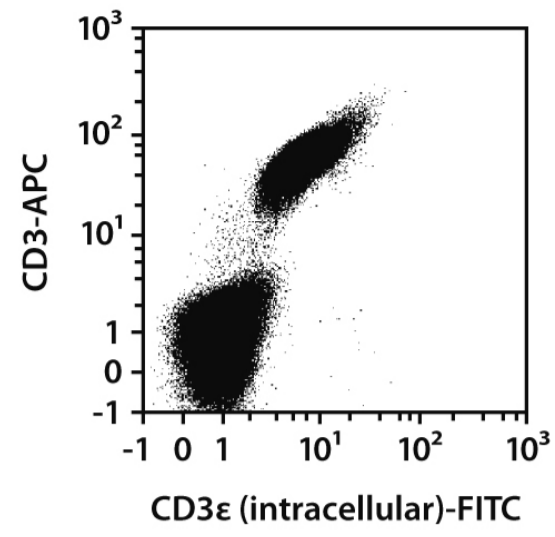
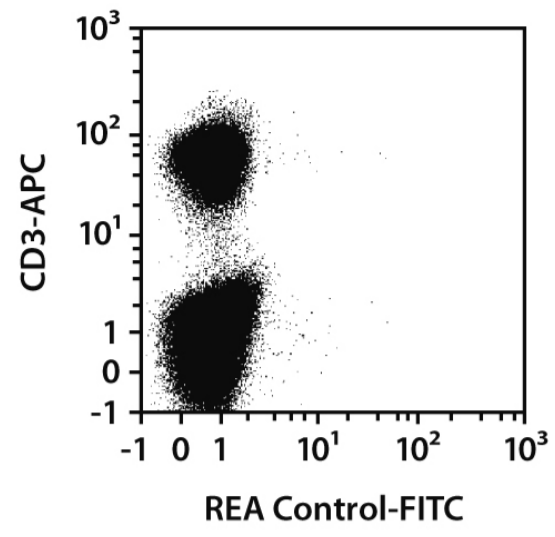
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other 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.
- Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells containing Inside Fix and Inside Perm.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

Protocol for intracellular staining of cells

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10⁶ cells/100 µL.
 - Volumes given below are for up to 10⁶ nucleated cells. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.
1. Wash up to 10⁶ cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
 2. (Optional) Stain cell surface antigens that are sensitive to fixation with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
 3. Resuspend up to 10⁶ cells in 250 µL of buffer.
 4. Add 250 µL of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes in the dark at room temperature.
 5. Centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
 6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
Note: Fixed cells may be stored in azide-containing buffer at 2–8 °C for up to 1 week.
 7. (Optional) Stain cell surface antigens that are sensitive to permeabilization with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
 8. Wash cells by adding 1 mL of Inside Perm (Inside Stain Kit) and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
 9. Resuspend cells in 98 µL of Inside Perm. Add 2 µL of the antibody.
Note: For staining with several antibodies in this step, reduce the volume of Inside Perm accordingly. For efficient permeabilization, the volume of Inside Perm should be at least 30% of the overall staining volume.
 10. Mix well and incubate for 10 minutes in the dark at room temperature.
 11. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
 12. (Optional) If biotinylated antibody was used, resuspend the cell pellet in Inside Perm and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
 13. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.
- Note: Samples may be stored at 2–8 °C in the dark for up to 24 hours.
 - Note: Do not use propidium iodide (PI) or 7-AAD staining.

Examples of immunofluorescent staining

Splenocytes from C57BL/6 mice were fixed, permeabilized, and stained with CD3ε (intracellular) antibodies or with the corresponding REA Control antibodies (left images) as well as with CD3 antibodies. Flow cytometry was performed using the MACSQuant[®] Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals.



Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | www.miltenyibiotec.com Miltenyi Biotec provides products and services worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. autoMACS, MACS, MACSQuant, Vio, VioBlue, VioBright, and VioGreen are either trademarks or registered trademarks of Miltenyi Biotec GmbH. Copyright © 2016 Miltenyi Biotec GmbH. All rights reserved.