CD8a 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.

Product	Content	Order no.
CD8a-FITC	9 µg in 300 µL	130-108-910
CD8a-FITC	30 µg in 1 mL	130-108-880
CD8a-PE	9 µg in 300 µL	130-108-911
CD8a-PE	30 µg in 1 mL	130-108-881
CD8a-APC	9 µg in 300 µL	130-108-912
CD8a-APC	30 µg in 1 mL	130-108-882
CD8a-VioBlue	9 µg in 300 µL	130-108-909
CD8a-VioBlue	30 µg in 1 mL	130-108-879
CD8a-VioGreen	9 µg in 300 µL	130-108-908
CD8a-VioGreen	30 µg in 1 mL	130-108-878
CD8a-PE-Vio770	9 µg in 300 µL	130-108-913
CD8a-PE-Vio770	30 µg in 1 mL	130-108-883
CD8a-APC-Vio770	9 µg in 300 µL	130-108-914
CD8a-APC-Vio770	30 µg in 1 mL	130-108-884
CD8a-PerCP-Vio700	9 µg in 300 µL	130-108-915
CD8a-PerCP-Vio700	30 µg in 1 mL	130-108-885
CD8a-Biotin	9 µg in 300 µL	130-108-907
CD8a-Biotin	30 µg in 1 mL	130-108-877

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	CD8a
Clone	REA437
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	OX-8, Lyt2, Ly-2
Molecular mass of antigen [kDa]	23
Distribution of antigen	NK cells, T cells, thymocytes, other
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 REA437 recognizes the rat CD8a antigen, a single-pass type I membrane protein, which is also known as OX-8. CD8 acts as a co-receptor for the T cell receptor and recognizes antigen displayed by an antigen presenting cell in the context of class I MHC molecules. CD8a is expressed as a heterodimer with CD8b on T cell receptor (TCR) α/β and TCR γ/δ expressing, cytotoxic T cells, and on most thymocytes. As a homodimer of two CD8a chains the CD8 antigen is expressed on most NK cells, a major fraction of intestinal intraepithelial lymphocytes, some activated $CD4^+CD8^+$ T cells and $CD8^+$ T cells from athymic rats. CD8a is also expressed by a T cell subset with regulatory functions. Additional information: Clone REA437 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

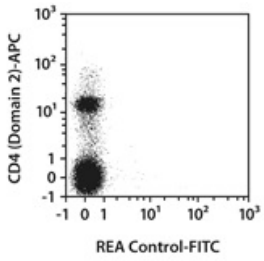
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

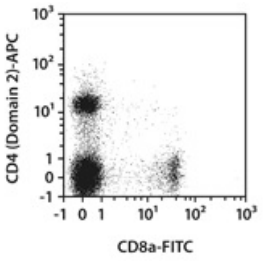
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 LOU rats were stained with CD8a antibodies or with the corresponding REA Control antibodies (left image) as well as with CD4 (Domain 2) 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.


CD4 (Domain 2)-APC
REA Control-FITC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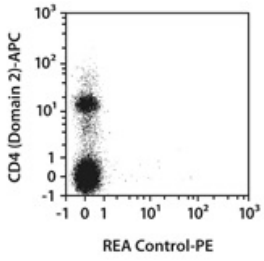
CD8a-FITC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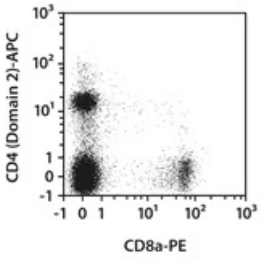
REA Control-PE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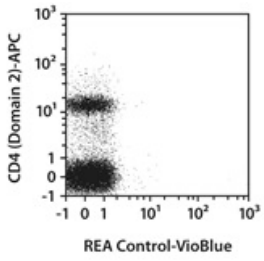
CD8a-PE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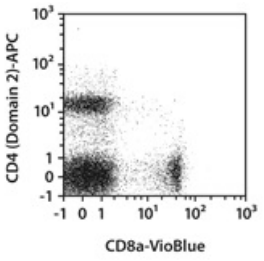
REA Control-VioBlue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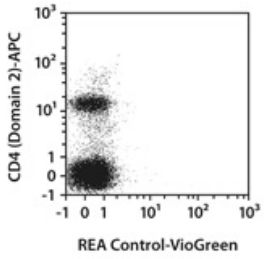
CD8a-VioBlue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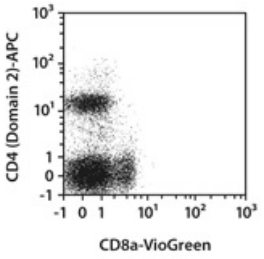
REA Control-VioGreen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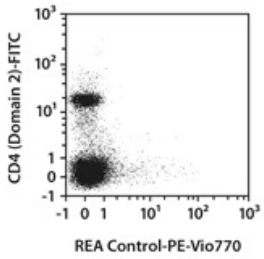
CD8a-VioGreen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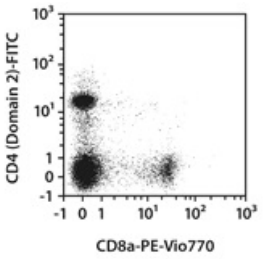
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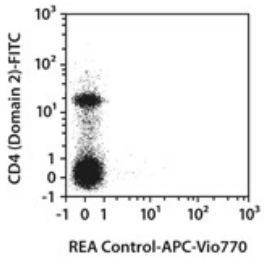
CD8a-PE-Vio770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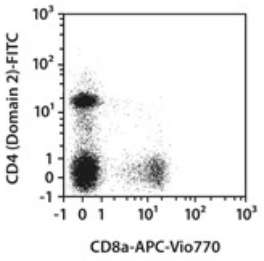
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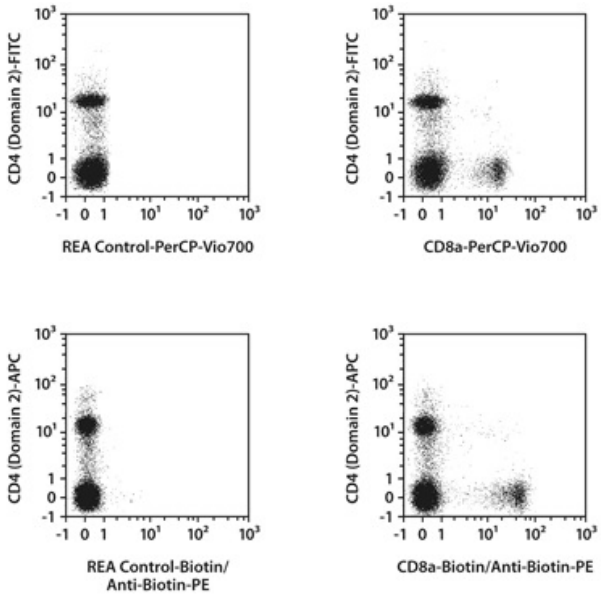

References

1. Johnson, P. *et al.* (1985) Purification, chain separation and sequence of the MRC OX-8 antigen, a marker of rat cytotoxic T lymphocytes. EMBO J. 4(10): 2539–2545.
2. **Torres-Nagel, N. *et al.*** (1992) Differential thymus dependence of rat CD8 isoform expression. Eur. J. Immunol. 22(11): 2841–2848.
3. **Strup-Perrot, C. *et al.*** (2005) Expression of matrix metalloproteinases and tissue inhibitor metalloproteinases increases in X-irradiated rat ileum despite the disappearance of CD8a T cells. World J Gastroenterol. 11(40): 6312–6321.

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