Miltenyi Biotec

REA Control (S) antibodies

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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.

1. Description

This product is for research use only.

Components Monoclonal REA Control (S) antibodies

Conjugate	Order no. 1 mL (100 tests)
FITC	130-104-610
VioBright™ FITC	130-104-576
PE	130-104-612
APC	130-104-614
VioBlue®	130-104-609
VioGreen™	130-104-608
PE-Vio® 615	130-107-146
PE-Vio770	130-104-616
APC-Vio770	130-104-618
PerCP-Vio700	130-104-620
Biotin	130-104-606

Clone REA293

Capacity 1 mL: 100 tests or up to 10^9 total cells

Product format Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

- Antigen: keyhole limpet hemocyanin (KLH)
- Expression patterns: The REA Control (S) antibody clone REA293 is a universal isotype control that can be used with all recombinant engineered antibodies (REAfinity™ Antibodies) that recognize cell surface antigens. REAfinity Antibodies have been engineered for their high specificity and contain human IgG1 parts for constant regions. Although REAfinity Antibodies show virtually no binding to Fc receptors, the use of the clone REA293 is still recommended to control for other non Fc receptor–mediated non-specific binding of fluorochrome-conjugated REAfinity Antibodies to cells. Unspecific interactions of the fluorochrome with the cell surface can also be controlled with conjugated versions of clone REA293.

1.2 Applications

- Universal control for all recombinant engineered antibodies (REAfinity Antibodies) that recognize surface antigens.

1.3 Recommended antibody dilution

The recommended antibody dilution for all REA Control (S) conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

1.4 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) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye–conjugated antibodies to human cells, especially to monocytes.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with REA Control (S)-Biotin.
- (Optional) For antibodies for additional staining, refer to www.miltenyibiotec.com/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.

140-004-505.03

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2. General protocol for immunofluorescent staining

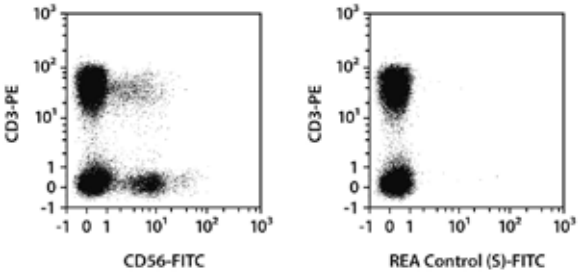
▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

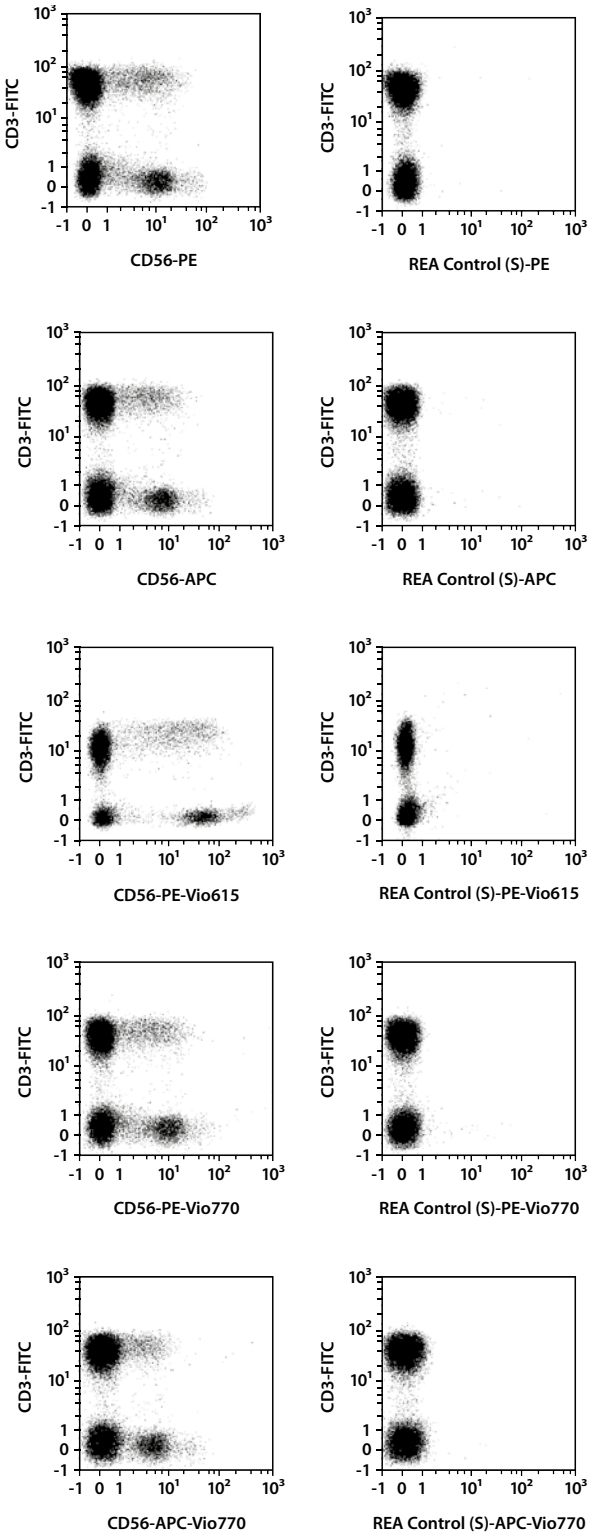
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the REA Control (S) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

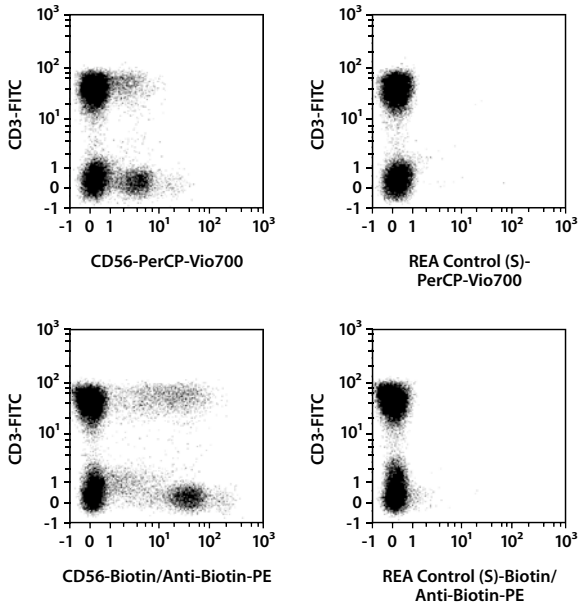
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If REA Control (S)-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of 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.

3. Example of immunofluorescent staining with REA Control (S) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with REA Control (S) antibodies or with the corresponding CD56 (clone REA196) 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 and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.







Refer to www.miltenyibiotec.com for all data sheets and protocols.

Warranty

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