Miltenyi Biotec

REAlease® Support Kit

Order no. 130-120-675

Contents

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.

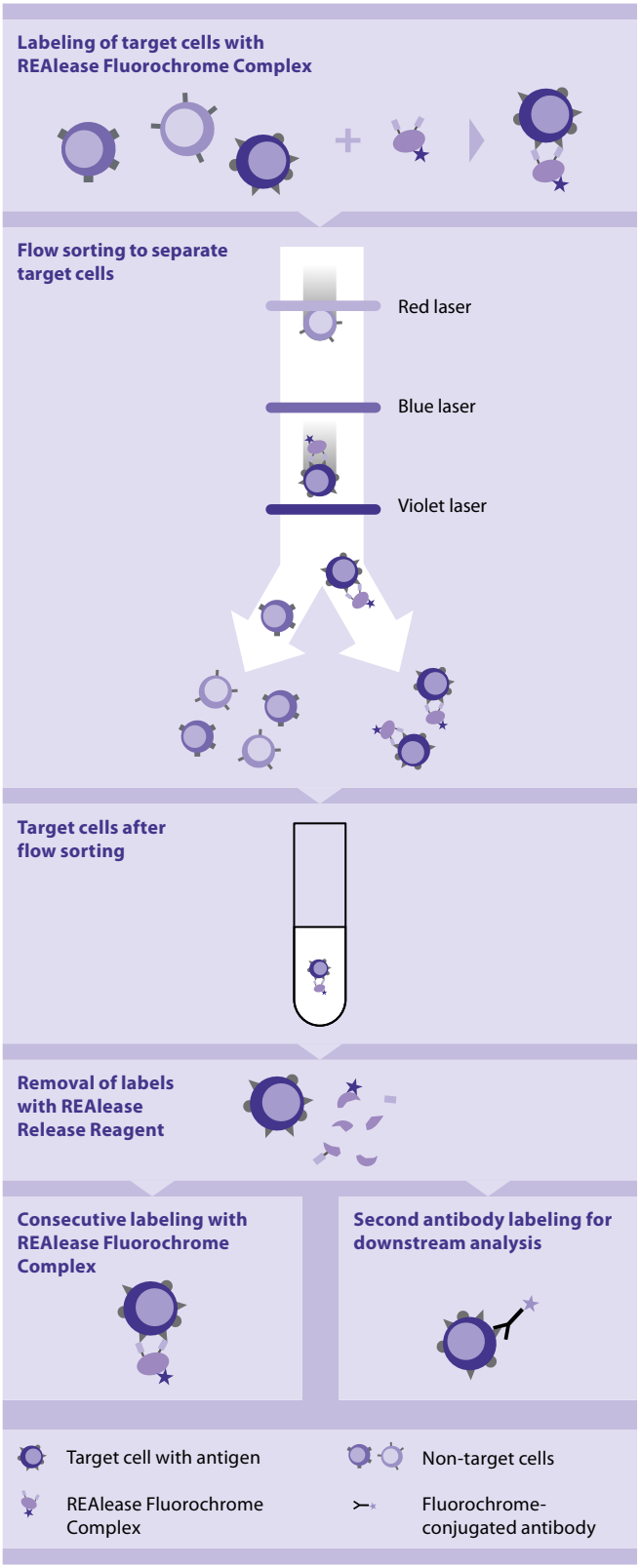
1. Description

This product is for research use only.

Components	**1 mL REAlease Release Reagent** **2× 1 mL REAlease Stop Reagent**
Capacity	For 5×10^8 total cells, up to 50 reactions.
Product format	All reagents are supplied in buffer containing 0.05% sodium azide. REAlease Release Reagent contains stabilizer.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the REAlease Fluorochrome Technology

The REAlease Fluorochrome Technology relies on recombinantly engineered antibody fragments (REAlease Complex) which are characterized by low epitope binding affinities and no binding to Fc receptors.
First, cells are labeled with a fluorchrome-conjugated REAlease Complex. Then, the REAlease Complex can be removed from the cell surface by the addition of the REAlease Release Reagent. Cells are washed using the REAlease Stop Reagent and can now be relabeled with different fluorochrome-conjugated REAlease Complexes.



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140-005-477.02

1.2 Applications

- Removal of fluorchrome-conjugated REAlease Complexes.

1.3 Reagent and instrument requirements

- Fluorchrome-conjugated REAlease Complexes, e.g., REAlease CD19-FITC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- 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.

2. Protocol for removal of REAlease Complexes

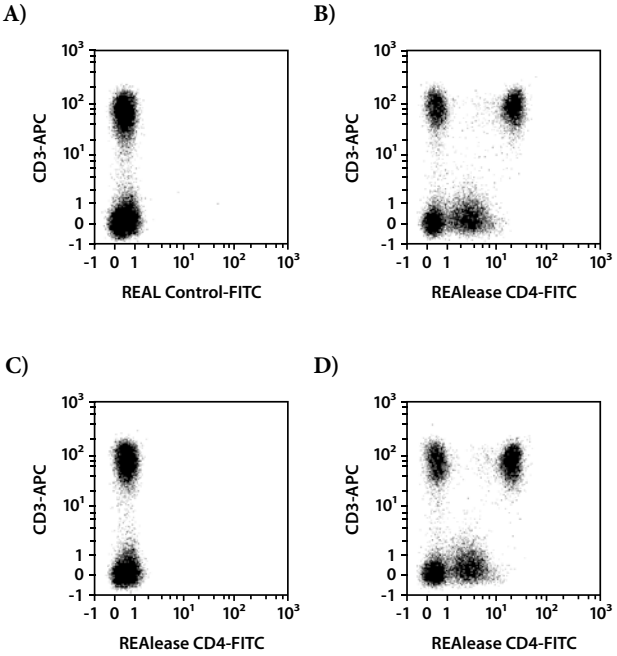
▲ Volumes given below are for up to 10^7 stained cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

▲ The recommended incubation temperature is room temperature. Lower temperatures may lead to decreased removal efficiency.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 cells in 980 µL of buffer.
4. Add 20 µL of REAlease Release Reagent.
5. Mix well and incubate for 10 minutes in the dark at room temperature.
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) For relabeling with REAlease Complexes resuspend the cell pellet in 1970 µL of buffer and add 30 µL of REAlease Stop Reagent. Mix well and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely. Resuspend up to 10^6 nucleated cells per 68 µL of buffer and add 30 µL of REAlease Stop Reagent. Mix well and add fluorchrome-conjugated REAlease Complexes according to the data sheet.
8. Resuspend cell pellet in a suitable amount of buffer or media for downstream application.

3. Example of immunofluorescent staining and removal of the REAlease Complex

Human peripheral blood mononuclear cells (PBMCs) were stained with REAlease CD4-FITC (B) or with the corresponding REAL Control (A). REAlease CD4-FITC was released using the REAlease Release Reagent (C). To show epitope accessibility after REAlease Complex release, the cells were washed with the REAlease Stop Reagent and restained with REAlease CD4-FITC (D). Cells were labeled with CD3 antibodies in addition and were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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