Miltenyi Biotec

REAlease® CD8a MicroBead Kit
mouse

Order no. 130-126-707

Contents

1. Description

This product is for research use only.

Components	**2×1 mL REAlease CD8a-Biotin, mouse** **2×5 mL REAlease Anti-Biotin MicroBeads (CD8a, mouse)** **2×4 mL REAlease Bead Release Reagent (50×)** **4 mL REAlease Release Reagent** **2×4 mL REAlease Stop Reagent**
Capacity	For 2×10^9 total cells, up to 100 separations.
Product format	REAlease Stop Reagent is supplied in buffer containing 0.05% sodium azide. All other reagents 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 Principle of the REAlease MACS Separation

The REAlease Technology relies on recombinantly engineered antibody fragments to label specific cell surface markers. The fragments are developed to have low affinity for epitopes. However, when fragments are multimerized as a REAlease Biotin Complex (i.e., REAlease CD8a-Biotin, mouse) they bind to epitopes with high avidity. REAlease Technology can control the multimer/monomer state of antibody fragments. With this technology a controlled label release is possible where monomerized antibody fragments dissociate from the cell surface, enabling users to obtain bead- and label-free cells.

First, the target cells are labeled with REAlease CD8a-Biotin (REAlease Biotin Complex). Subsequently, REAlease Anti-Biotin MicroBeads (CD8a, mouse) bind to the REAlease Biotin Complex. Then, the cell suspension is loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled cells are retained within the column. The unlabeled non-target cells flow through; this cell fraction is thus depleted of $CD8a^+$ cells. Target cells are eluted from the column using the REAlease Bead Release Reagent, which simultaneously removes the MicroBeads from the cells. For highest recovery, the column is removed from the magnetic field and target cells are eluted. For highest purity, target cells are eluted within the magnetic field of the column. Finally, during the subsequent incubation with the REAlease Release Reagent, the REAlease Biotin Complex monomerizes and dissociates from the cell surface leaving the cells free of all labels.

1.2 Background information

The REAlease CD8a MicroBead Kit, mouse has been developed for positive selection of mouse $CD8a^+$ cells from single-cell suspensions of lymphoid and non-lymphoid tissues. The CD8a antigen is expressed on most thymocytes, almost all cytotoxic T cells and on subpopulations of dendritic cells. CD8a functions as an accessory molecule in the recognition of MHC class I/peptide complexes by the TCR heterodimer on cytotoxic $CD8a^+$ T cells.

1.3 Applications

- Positive selection of mouse $CD8a^+$ cells from single-cell suspensions, e.g., from lymph nodes or lung preparations.

  ▲ **Note:** For separation of $CD8a^+$ T cells from spleen, the use of the StraightFrom® Spleen CD8a MicroBead Kit, mouse (# 130-130-149) or the $CD8a^+$ T Cell Isolation Kit, mouse (# 130-104-075) is recommended.

- Isolation of $CD8a^+$ label-free cells.

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1.4 Reagent and instrument requirements

- Separation 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). Use buffer at room temperature (19–25 °C). Store buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- REAlease Bead Release buffer: Prepare a 1:50 dilution of REAlease Bead Release Reagent (50×), e.g., for 1 mL add 20 µL of REAlease Bead Release Reagent to 980 µL of separation buffer.

  ▲ **Note:** Use freshly prepared buffer the same day. Store at room temperature.

  ▲ **Note:** For highest recovery protocol, prepare 1 mL per MS Column and 5 mL per LS Column. For highest purity protocol, prepare 3 mL per MS Column and 14 mL per LS Column.

- MACS Columns and MACS Separators: $CD8a^+$ cells can be enriched by using MS or LS Columns. Positive selection can also be performed by using autoMACS Columns on the autoMACS NEO Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS NEO Separator

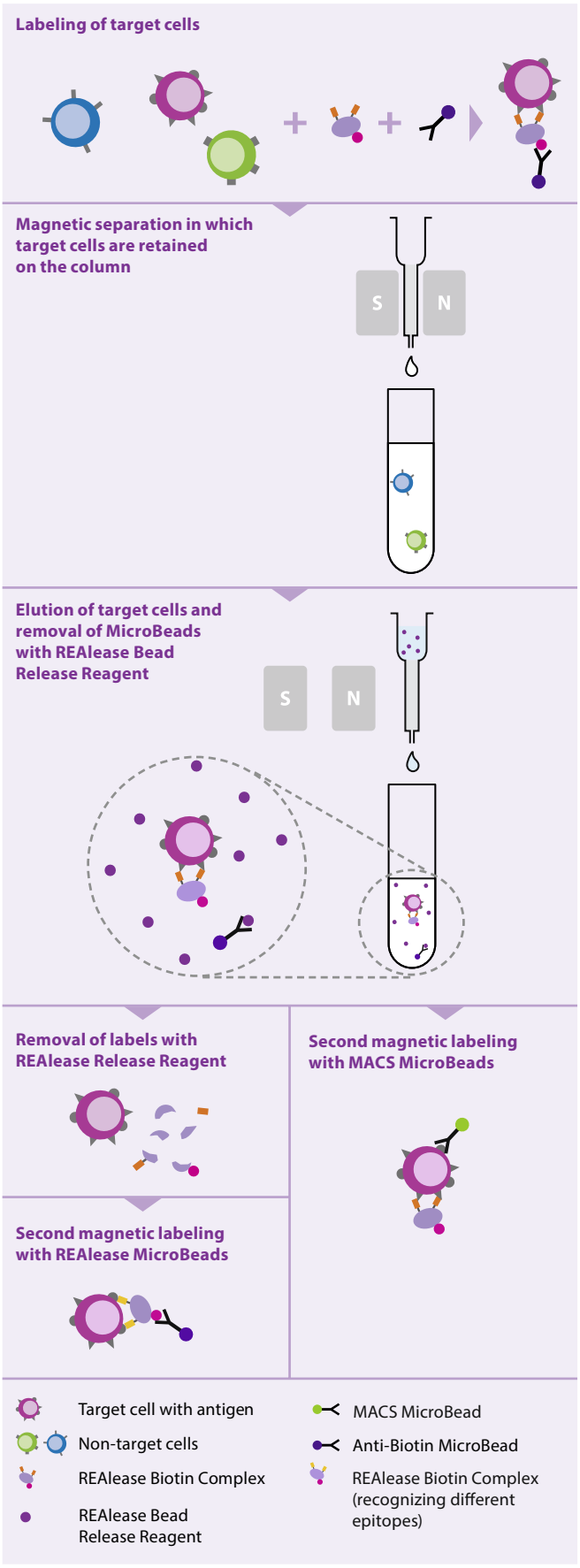
  ▲ **Note:** Column adapters are required to insert certain columns into SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45 Antibody, anti-mouse, VioGreen™ and CD8a Antibody, anti-mouse, APC-Vio® 770. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233), 7-AAD Staining Solution (# 130-111-568), or DAPI Staining Solution (# 130-111-570) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

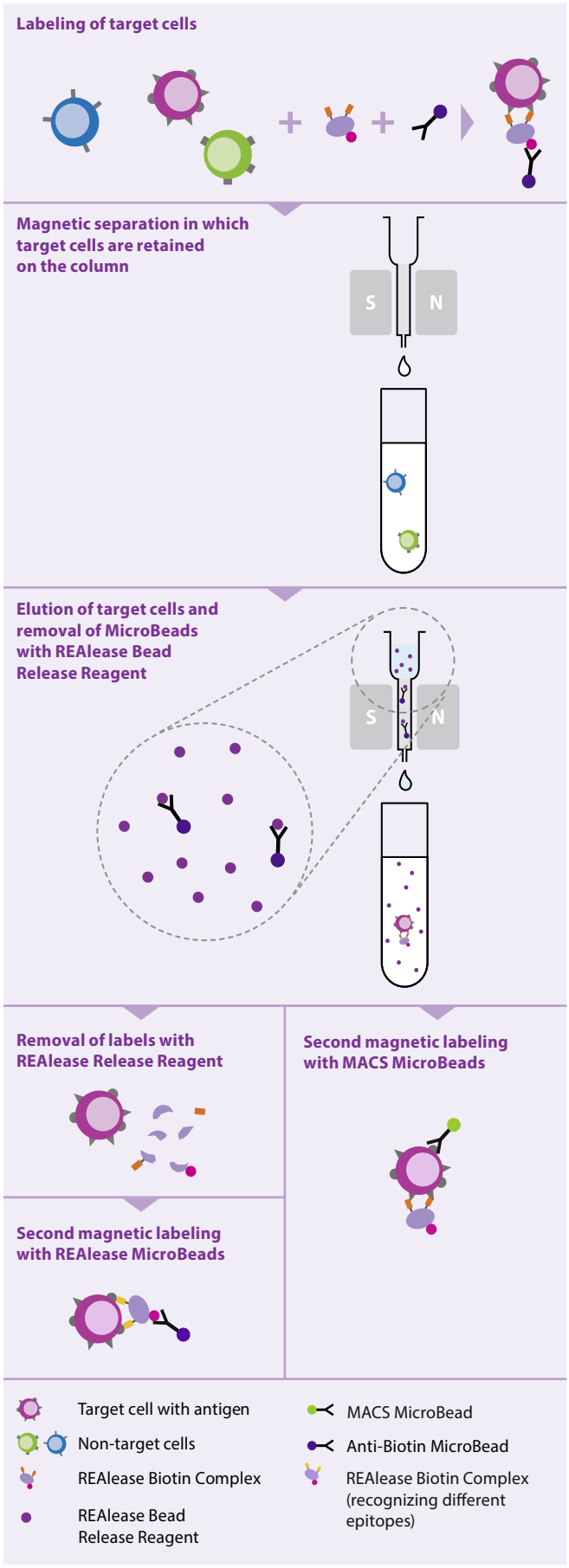
2. Protocol

2.1 Protocol overview

A) Highest recovery



B) Highest purity



2.2 Sample preparation

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.miltenyibiotec.com/gentlemacs.

2.3 Magnetic labeling

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, it is recommended to use density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

▲ The recommended incubation temperature is at room temperature (19–25 °C).

▲ Volumes for magnetic labeling given below are for up to 2×10^7 total cells. When working with fewer than 2×10^7 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 4×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of separation buffer per 2×10^7 total cells.
4. Add 20 µL of REAlease CD8a-Biotin per 2×10^7 total cells.
5. Mix well and incubate for 5 minutes.
6. Add 100 µL of REAlease Anti-Biotin MicroBeads (CD8a, mouse) per 2×10^7 total cells.
7. Mix well and incubate for 5 minutes.
8. (Optional) Add staining antibodies, e.g., CD45 Antibody, anti-mouse, VioGreen and CD8a Antibody, anti-mouse, APC-Vio 770, and incubate according to manufacturer's recommendation.
   ▲ **Note:** These staining antibodies cannot be removed from the cells.
9. Dilute up to 5×10^7 cells in a total volume of 500 µL with separation buffer.
   ▲ **Note:** For volumes larger than 500 µL a dilution is not needed.
10. Proceed to magnetic separation (2.4).

2.4 Magnetic separation and removal of magnetic labeling

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD8a^+$ cells. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

▲ The recommended incubation temperature is at room temperature (19–25 °C).

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of separation buffer:

   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of separation buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Proceed either to 2.4.1 if highest recovery is needed or 2.4.2 if highest purity is needed.

2.4.1 Highest recovery

6. Remove column from the separator and place it on a suitable collection tube.
7. (Optional) If removal of MicroBeads is not required, pipette appropriate amount of separation buffer. Immediately flush out target cells by firmly pushing the plunger into the column. Eluted cells are ready for downstream applications, e.g. flow cytometry analysis.

   MS: 1 mL LS: 5 mL
8. For removal of MicroBeads proceed with step 9.
9. Pipette the appropriate amount of REAlease Bead Release buffer (prepared by REAlease Bead Release Reagent (50×), refer to chapter 1.4) onto the column. Immediately flush out target cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL
10. Mix well and incubate for 10 minutes.
11. Cells are now free from MicroBeads and ready for analysis and downstream applications.
12. (Optional) Proceed either to
    - 2.6 Removal of REAlease Complex and second magnetic labeling with REAlease MicroBeads

      or proceed to
    - 2.7 Second magnetic labeling with MACS MicroBeads.

2.4.2 Highest purity

6. Place a new tube under the column to collect target cells
7. Pipette the appropriate amount of REAlease Bead Release buffer (prepared by REAlease Bead Release Reagent (50×), refer to chapter 1.4) onto the column. Collect flow-through containing target cells.

   MS: 3×1mL LS: 2×4 mL + 1×6 mL
8. Mix well and incubate for 10 minutes.
9. Cells are now free from MicroBeads and ready for analysis and downstream applications.
10. (Optional) Remove column from the separator and place it on a suitable collection tube. Apply separation buffer onto the column. Immediately flush out cells sticking on the column by firmly pushing the plunger into the column.

    MS: 1 mL LS: 5 mL
11. (Optional) Proceed either to
    - 2.6 Removal of REAlease Complex and second magnetic labeling with REAlease MicroBeads

      or proceed to
    - 2.7 Second magnetic labeling with MACS MicroBeads.

2.5 Magnetic labeling and separation using autoMACS Separators

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

▲ Buffers used for operating the autoMACS Separators should have a temperature of ≥10 °C.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = unlabeled (negative) fraction, position C = labeled (positive) fraction.

2.5.1 Magnetic labeling and separation using the autoMACS NEO Separator

▲ The autoMACS NEO Separator enables stage loading to extend column capacity for selected reagents, minimizing the need to divide larger samples.

▲ For more information on selecting alternative separation programs, stage loading–compatible reagents, autolabeling-compatible reagents, and the minimal and maximal volumes for each reagent and Chill Rack, refer to www.miltenyibiotec.com/automacs-neo-sample-processing.

2.6 (Optional) Removal of the REAlease Complex and second magnetic labeling with REAlease MicroBeads

▲ The recommended incubation temperature is at room temperature (19–25 °C).

▲ For second magnetic labeling with MACS Anti-Biotin MicroBeads proceed through all steps of chapter 2.6 to remove the REAlease Biotin Complex.

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2.6.1 Removal of the REAlease Complex

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in appropriate amount of separation buffer:
   MS: 1 mL LS: 5 mL
3. Add an appropriate amount of REAlease Release Reagent:
   MS: 20 µL LS: 100 µL
4. Mix well and incubate for 5 minutes.
5. Cells are now free from REAlease Complex and MicroBeads and are ready for analysis or downstream applications.
6. (Optional) For second magnetic labeling with REAlease MicroBeads continue with 2.6.2.

2.6.2 Second magnetic labeling with REAlease MicroBeads

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 40 µL of REAlease Stop Reagent per 10^7 total cells.
3. Mix well.
4. For a second magnetic labeling follow the labeling protocol in the respective REAlease MicroBead Kit data sheet.

   ▲ **Note:** For best recovery and purity of cells, the amount of MACS MicroBeads for the second positive labeling may need optimization as the starting frequency of target cells may be different from a PBMC sample.

2.7 (Optional) Second magnetic labeling with MACS MicroBeads

▲ For second magnetic labeling with MACS Anti-Biotin MicroBeads proceed through all steps of chapter 2.6 to remove the REAlease Biotin Complex.

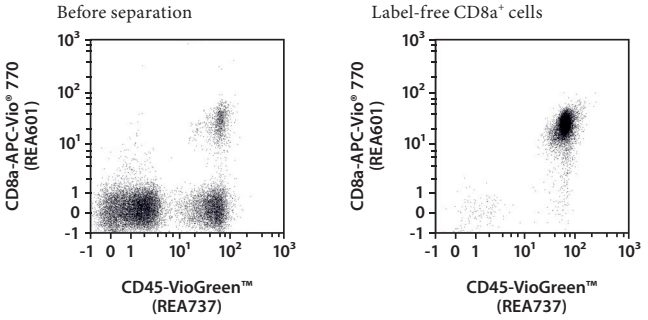
1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Add the recommended amount of MACS MicroBeads to label the cells magnetically for the second marker. For details refer to the respective MACS MicroBeads data sheet.

   ▲ **Note:** For best recovery and purity of cells, the amount of MACS MicroBeads for the second positive labeling may need optimization as the starting frequency of target cells may be different from a PBMC sample.

3. Example of a separation using the REAlease CD8a MicroBead Kit

A perfused mouse lung sample was dissociated using the gentleMACS Octo Dissociator with Heaters in combination with the Lung Dissociation Kit, mouse (# 130-095-927). $CD8a^+$ cells were isolated from the single-cell suspension using the REAlease CD8a MicroBead Kit, MS Columns, and an OctoMACS™ Separator using the highest recovery protocol. Cells were fluorescently stained with CD45-VioGreen and CD8a-APC-Vio 770 and analyzed by flow cytometry using the MACSQuant® Analyzer 10. Cell debris and dead cells were excluded from the analysis based on scatter signals and DAPI fluorescence.

A) Cell purity

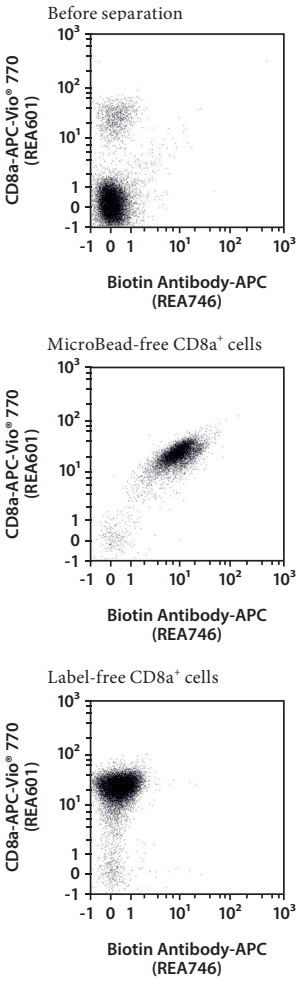


B) Bead-free cells: efficiency of REAlease Anti-Biotin MicroBeads release

Release efficiency was higher than 95% for the REAlease Anti-Biotin MicroBeads (CD8a). The efficiency was determined by re-applying the isolated cells to a second MACS Column. The ratio between the numbers of cells in the flow-through and the total number of cells applied to the second column allowed us to calculate the efficiency of magnetic labeling removal.

C) Label-free cells: REAlease Biotin Complex release

The efficient removal of all labels was shown by using Anti-Biotin-APC to analyze the cells by flow cytometry for the presence of REAlease Biotin Complex. Directly after isolation, the cells showed staining of biotin ("MicroBead-free $CD8a^+$ cells"), whereas the label-free $CD8a^+$ cells after the REAlease Biotin Complex release were negative for biotin similar to the non-labeled cells before separation.



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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