Dead Cell Removal Kit

Order no. 130-090-101

Contents

1. Description

This product is for research use only.

Components	**10 mL Dead Cell Removal MicroBeads** **25 mL 20× Binding Buffer Stock Solution**
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Dead Cell Removal MicroBeads are supplied in buffer containing stabilizer.
Storage	Store protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS Separation

Dead Cell Removal MicroBeads recognize a moiety in the plasma membrane of apoptotic as well as dead cells. For dead cell depletion, cells are magnetically labeled with Dead Cell Removal MicroBeads and passed through a separation column. The magnetically labeled dead cells are retained within the column. The unlabeled living cells run through; this cell fraction is thus depleted of dead cells. After removing the column from the magnetic field, the magnetically retained dead cells can be eluted as the positively selected cell fraction. Using the Dead Cell Removal Kit, even early apoptotic cells with an intact cellular membrane are removed. Activated cells, e.g., from a cell culture, may be labelled as well.

1.2 Reagent and instrument requirements

- Sterile, double-distilled water (ddH_2O).

  ▲ **Note:** Do not use deionized water for dilution.

- MACS Columns and MACS Separators: Dead cells can be depleted by using MS, LS, XS, or D Columns or depleted with the use of D Columns. Depletion can also be performed by using autoMACS Columns on the autoMACS NEO or autoMACS Pro Separators.

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

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

- (Optional) MACS BSA Stock Solution (# 130-091-376).
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), gentleMACS Dissociator with Heaters (# 130-096-427).
- (Optional) MACS Tissue Dissociation Kits, e.g. Tumor Dissociation Kit, mouse (# 130-096-730).

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2. Protocol

2.1 Overview

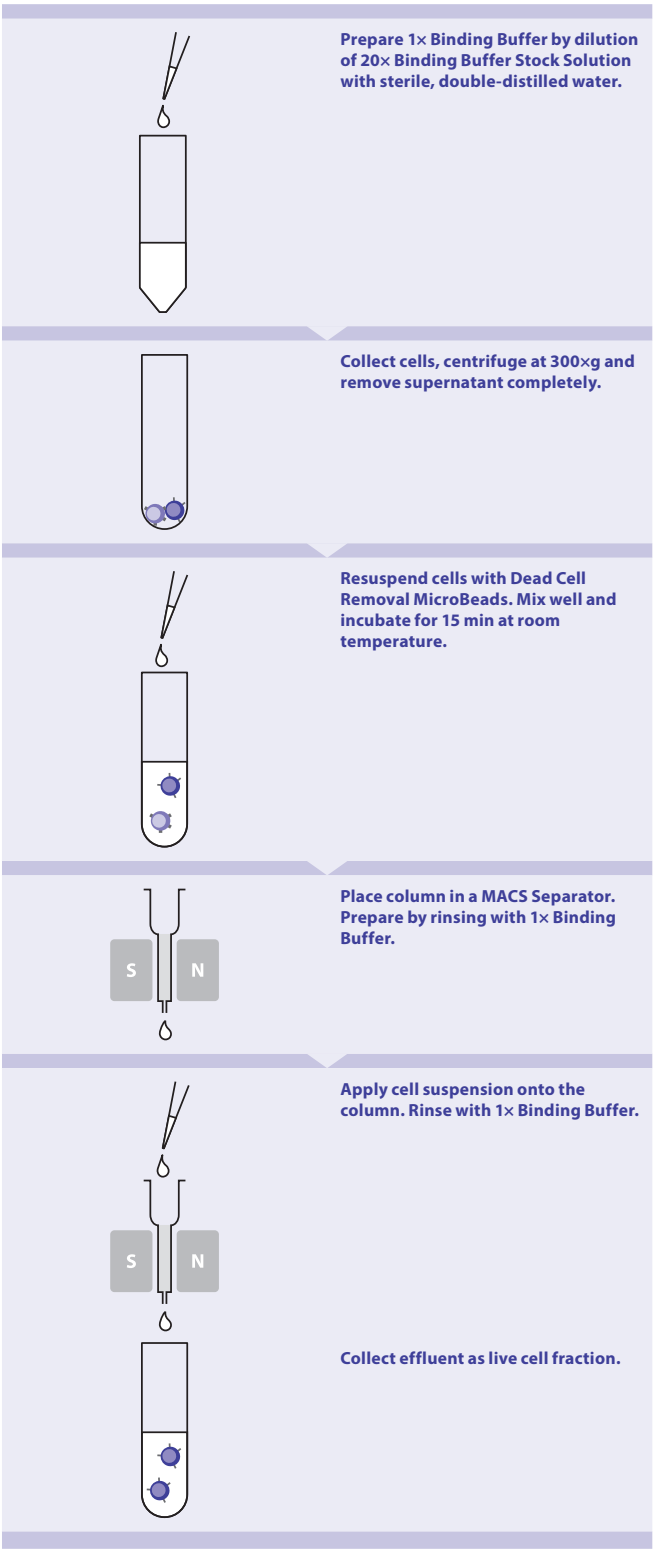

Figure 1: Elimination of dead cells using the Dead Cell Removal Kit.

2.2 Sample preparation

▲ Dead Cell Removal MicroBeads are susceptible to bacterial contamination. Handle under sterile conditions.

▲ When working with cell samples containing platelets, e.g., blood samples, wash samples carefully at low centrifugation speed (200×g) in order to remove platelets. Use buffer containing the ion chelator EDTA for these washing steps. Dead Cell Removal MicroBeads bind to activated platelets. Activated platelets also bind to leukocytes, e.g., monocytes. In this case, viable cells bound to activated platelets would be retained in the magnetic field and reduce the recovery of living cells.

When working with tissues, prepare a single-cell suspension using gentleMACS Dissociators and MACS Tissue Dissociation Kits.

▲ Dead cells without any remnants of the plasma membranes (cell organelles without nuclei) cannot be removed using Dead Cell Removal MicroBeads due to lack of accessible antigen.

2.3 Buffer preparation

▲ Use 1× Binding Buffer prepared from 20× Binding Buffer Stock Solution supplied with the Dead Cell Removal Kit for all washing and selection steps. It is important to use only sterile double-distilled water for the dilution of the 20× Binding Buffer Stock Solution.

▲ **Note:** Do not use deionized water for dilution.

Prepare 1× Binding Buffer from 20× Binding Buffer Stock Solution. e.g., dilute 500 µL of 20× Binding Buffer Stock Solution with 9.5 mL of sterile, double-distilled water. Store at +2 to +8 °C. Alternatively, prepare 1× Binding Buffer Stock Solution by diluting 25 mL of 20× Binding Buffer with 475 mL of sterile, double-distilled water. It is recommended to add 1% BSA, e.g., MACS BSA Stock Solution (# 130-091-376), to increase separation efficiency.

▲ **Note:** Handle under sterile conditions!

▲ **Note:** Binding of Dead Cell Removal MicroBeads requires Ca^{2+}. The presence of the ion chelator EDTA will abolish binding. 1× Binding Buffer is optimized for best Dead Cell Removal MicroBeads binding. The use of a different buffer may lead to poor dead cell removal efficiency.

2.4 Magnetic labeling

▲ Volumes for magnetic labeling given below are for up to 10^7 total 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 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.

▲ The recommended incubation temperature is +20 to +25 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

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3. Resuspend cell pellet in 100 µL of Dead Cell Removal MicroBeads per 10^7 total cells.
4. Mix well and incubate for 15 minutes at room temperature (+20 to +25 °C).
5. (Optional) If necessary, add 1× Binding Buffer to the cell suspension to reach a minimum volume of 500 µL for separation.
8. Proceed to magnetic separation (2.5).

2.5 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of labeled cells. For details refer to the table in section 1.2.

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

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 1× Binding Buffer:

   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells (live cells).
4. Wash column with the appropriate amount of 1× Binding Buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   MS: 4×500 µL LS: 4×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL
7. (Optional) To increase the efficiency of magnetic removal of dead cells, the live cell fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.
8. Proceed to microscopic analysis of dead cells with membrane exclusion dyes, such as Trypan Blue or to flow cytometric analysis using Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568).

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

2.6 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.

2.6.1 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.

Magnetic separation after manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Place the Chill Rack on the MACS MiniSampler S.
4. Select the same Chill Rack in the **Experiment** tab. An experiment is created automatically. Tap to select sample positions.
5. Assign a reagent to each sample.
6. Manual labeling is set automatically if autolabeling is not supported or no reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Manual**.
7. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.

8 The separation program for highest target cell purity is selected by default. Refer to the **Sample process** pane for all available programs.

9. Place the sample(s) and empty tubes to the Chill Rack.
10. Tap **Run** to start the separation process.

2.6.2 Magnetic labeling and separation using the autoMACS Pro Separator

Magnetic separation after manual labeling

▲ Refer to the respective user manual for instructions on how to use the autoMACS Pro Separator.

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

▲ The program "Depls_b" works only **with manual labeling** and only with the Chill 15 Rack. Perform manual labeling according to chapter 2.4 until step 4.

▲ Elution of the dead cells (labeled cells) is optional.

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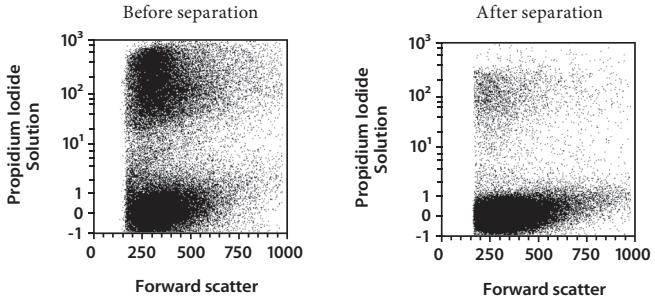
▲ The program "Depls_b" contains a 15-minute incubation timer before the separation. A countdown will be shown on the display when the run is started, and the separation will follow immediately after. This enables the incubation of the samples on the instrument, but it applies only once before starting the separation. To omit the incubation on the instrument, press **Skip**.

1. Prepare and prime the instrument.
2. Apply tube containing the sample in row A of the tube rack. Place tube to collect the unlabeled cell fraction (live cells) in row B and and provide a tube containing 10 mL of 1× Binding Buffer in row C.
3. For a standard separation choose the following program:

   Depletion: Depls_b
   Collect unlabeled cell fraction in row B of the tube rack. This fraction represents the live cells.
4. Use the wash program **Clean**.
5. (Optional) For elution of dead cells (labeled cells), press **Pos**. Cells will be eluted in tube containing 1× Binding Buffer in row C. At this step, remaining volume of 1× Binding Buffer in the tube will be 0.5mL. To discard dead cells, press **Waste**.

3. Example for elimination of dead cells from tissue using the Dead Cell Removal Kit

Dead cells were eliminated from peripheral blood mononuclear cells (PBMCs) subjected to heat-shock by using the Dead Cell Removal Kit, an LS Column, and a MidiMACS™ Separator. Dead cells were fluorescently stained with propidium iodide and analyzed using a MACSQuant® Analyzer 10.



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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