Miltenyi Biotec

Cell Suspension Cleanup Kit
human

Order no. 130-135-177

Contents

1. Description

This product is for research use only.

Components	**10 mL Cleanup Cocktail A, human** **1 mL Cleanup Cocktail B, human** **25 mL 20× Binding Buffer Stock Solution**
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Cleanup Cocktail A, human and Cleanup Cocktail B, human 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

MicroBeads from Cleanup Cocktail A, human and Cleanup Cocktail B, human recognize a moiety in the plasma membrane of apoptotic, dead cells, red blood cells (RBCs), and cell-free organelles. For depletion of unwanted cells and cell-free organelles, cells and organelles are magnetically labeled with MicroBead Cocktails and passed through a separation column. The magnetically labeled unwanted cells and organelles are retained within the column. The unlabeled living cells run through. After removing the column from the magnetic field, the magnetically retained dead cells, RBCs, and cell-free organelles can be eluted as the positively selected fraction. Using the Cell Suspension Cleanup Kit, human even early apoptotic cells with an intact cellular membrane are removed. Activated cells, for example from a cell culture, may be labeled as well.

Please note, that the Cell Suspension Cleanup Kit, human is not compatible with cell suspensions that have previously been treated with papain. This includes cell suspensions that have been dissociated using MACS Tissue Dissociation Kits containg Enzyme P. Papain and Enzyme P degrade an essential epitope required for the effective removal of erythrocytes by the kit. As an alternative workflow, the Red Blood Cell Lysis Solution (10×) (# 130-094-183) in combination with the Dead Cell Removal Kit (# 130-090-101) can be used.

1.2 Reagent and instrument requirements

- Sterile, double-distilled water (ddH_2O).
  ▲ **Note:** Do not use deionized water for dilution.
- LS Columns (# 130-042-401)
- MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator
- MACS MultiStand (# 130-042-303)
- (Optional) MACS BSA Stock Solution (# 130-091-376)
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.
- (Optional) gentleMACS™ Octo Dissociator with Heaters or gentleMACS Dissociator
- (Optional) gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) MACS Tissue Dissociation Kits without Enzyme P, e.g., Tumor Dissociation Kit, human (# 130-095-929)

2. Protocol

▲ Assure that cell suspensions are free of papain.

▲ Use pre-cooled MACS Separators for separation. Pre-cool MACS Separators for at least for 2 hours at +2 to +8 °C.

2.1 Sample preparation

▲ Cleanup Cocktail A, human and Cleanup Cocktail B, human are susceptible to bacterial contamination. Handle under sterile conditions.

▲ Prepare appropriate aliquots of Cleanup Cocktail A, human and Cleanup Cocktail B, human.

▲ 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. Cleanup Cocktail A, human binds to activated platelets. Activated platelets also bind to leukocytes, e.g., monocytes. In this case, viable cells bound to activated platelets are be retained in the magnetic field and reduce the recovery of living cells.

▲ **Note:** After washing with buffer containing EDTA, ensure to remove EDTA with additional washing steps with buffer without EDTA before proceeding with the protocol.

▲ When working with tissues, prepare a single-cell suspension using gentleMACS Dissociators and MACS Tissue Dissociation Kits without Enzyme P.

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2.2 Buffer preparation

▲ Use 1× Binding Buffer prepared freshly from 20× Binding Buffer Stock Solution supplied with the Cell Suspension Cleanup Kit, human for all washing and selection steps. Use only sterile double-distilled water for the dilution of the 20× Binding Buffer Stock Solution. 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. For higher cell recovery it is recommended to supplement the freshly prepared 1× Binding Buffer with 1% RNAse-free BSA. Store at +2 to +8 °C.

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

▲ **Note:** Binding of Cleanup Cocktail A, human requires Ca^{2+}. The presence of the ion chelator EDTA will abolish binding. The use of a different buffer may lead to poor efficiency.

2.3 Magnetic labeling

▲ Use pre-cooled reagents and buffers only.

▲ 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 +2 to +8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number including RBCs.
2. Centrifuge cell suspension at 300×g for 10 minutes at +2 to +8 °C. Aspirate supernatant completely.
3. Resuspend cell pellet in 100 µL of Cleanup Cocktail A, human per 10^7 total cells.
4. Add 10 µL of Cleanup Cocktail B, human per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (+2 to +8 °C).
6. (Optional) If necessary, add 1× Binding Buffer to the cell suspension after labeling to reach a minimum volume of 500 µL for separation.
7. Proceed to magnetic separation (2.4).

2.4 Magnetic separation with LS Columns

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

1. Place a LS Column in the magnetic field of a suitable pre-cooled MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with 3 mL of 1× Binding Buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing enriched viable cells in a fresh tube placed on ice.
4. Wash column with 2×1 mL of 1× Binding Buffer. Collect flow-through containing unlabeled cells, representing enriched viable cells and combine it with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. (Optional) Remove column from the separator and place it on a suitable collection tube.
6. Pipette 3 mL of 1× Binding Buffer onto the column. Immediately flush out the magnetically labeled dead cells, RBCs, and cell-free organelles by firmly pushing the plunger into the column.
7. (Optional) To increase the efficiency of magnetic removal of dead cells, RBCs, and cell-free organelles, the live cell fraction can be enriched over a second column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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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