Dead Cell Removal Kit

10 mL Dead Cell Removal MicroBeads
25 mL 20× Binding Buffer Stock Solution

Order No. 130-090-101

Cell Isolation Kits

Components of MACS® Dead Cell Removal Kit

- MACS® colloidal super-paramagnetic Dead Cell Removal MicroBeads. The product is supplied as a suspension containing stabilizer.
- MACS 20× Binding Buffer stock solution. The buffer has to be diluted 20-fold with sterile, double distilled water prior to use (see Preparation of 1× Binding Buffer).

These MACS products are susceptible to bacterial contamination. **Handle under sterile conditions!**

Storage Conditions

Store protected from light at 4 °C. Do not freeze.

Instruments Required

Magnetic cell separators MiniMACS™, MidiMACS™, VarioMACS™ or SuperMACS™.
MACS Column(s) MS or LS (plus MS or LS adapter for use in combination with VarioMACS or SuperMACS).

Protocol for Dead Cell Removal

Preparation of 1× Binding Buffer

Per 10^7 **total** cells, dilute 0.25 mL of 20× Binding Buffer Stock Solution with 4.75 mL of sterile, double distilled water. Alternatively, the total amount of 25 mL of 20× Binding Buffer Stock Solution can be diluted with 475 mL of sterile, double distilled water. Store at 4 °C. **Important: Handle under sterile conditions!**

Magnetic labeling

Collect cells e.g. from cell culture.

Centrifuge cells at 300×g. Remove supernatant completely and resuspend cell pellet in 100 µL of Dead Cell Removal MicroBeads per approximately 10^7 **total** cells (e.g. petri dish Ø 9 cm: about 1×10^7 cells, one mouse spleen: about 1×10^8 cells). For fewer cells, use same volume. Mix well and incubate for 15 minutes at **room temperature** (20–25 °C).

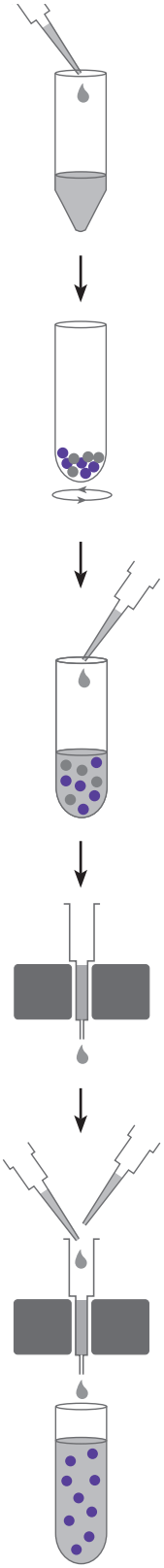
Preparation of MACS® Column

Choose a positive selection column type MS (for up to 10^7 **dead** cells and up to 2×10^8 **total** cells) or LS (for up to 10^8 **dead** cells and up to 2×10^9 **total** cells) and place the column in the magnetic field of a suitable MACS® Separator (see "Column Data Sheets").

Prepare column by rinsing with 1× Binding Buffer (MS: 500 µL; LS: 3 mL; for details, see "Column Data Sheets").

Magnetic separation

Apply cell suspension in suitable amount of 1× Binding Buffer onto the column (MS: 500-1000 µL; LS: 1–10 mL; see "Important Notes"). Let the negative cells pass through. Rinse with appropriate amount of 1× Binding Buffer (MS: 4×500 µL; LS: 4×3 mL). Collect effluent as live cell fraction.



How to eliminate dead cells using Dead Cell Removal Kit

1. Prepare 1× Binding Buffer by dilution of 20× Binding Buffer Stock Solution.
2. Collect cells, centrifuge at 300×g and remove supernatant completely.
3. Resuspend cells in Dead Cell Removal MicroBeads. Mix well and incubate for 15 min at room temperature.
4. Place column in MACS Separator, prepare by rinsing with 1× Binding Buffer.
5. Apply cell suspension onto the column. Rinse with 1× Binding Buffer.
6. Collect effluent as live cell fraction.

130-090-119.04

Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

Important Notes

- To increase the efficiency of the magnetic removal of dead cells, the live cell fraction can be passed over a second, freshly prepared column. Alternatively, the whole procedure (Magnetic Labeling and Magnetic Separation) can be repeated.
- Dead Cell Removal MicroBeads are susceptible to bacterial contamination. **Handle under sterile conditions**.
- Use 1× Binding Buffer prepared from 20× Binding Buffer Stock Solution supplied with the Dead Cell Removal Kit for all magnetic labeling, washing and selection steps. 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.
- **Attention:** Working on ice or incubating in the refrigerator requires increased incubation times for MACS MicroBeads. Incubate at **room temperature** (20–25 °C).
- Dead cells without any remnants of the plasma membranes ("stripped" nuclei) cannot be removed using Dead Cell Removal MicroBeads due to lack of accessible antigen.
- 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.

Background Information

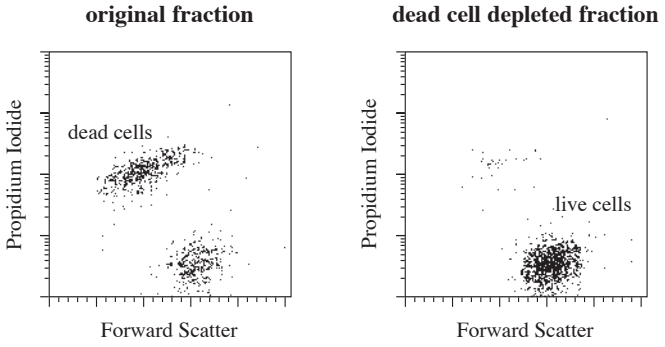
Dead Cell Removal MicroBeads recognize an antigen in the plasma membrane of apoptotic as well as dead cells. For the MACS dead cell depletion, cells are magnetically labeled with Dead Cell Removal MicroBeads and passed through a separation column. The magnetically labeled dead cells are retained in the column while the unlabeled living cells are collected in the flow-through. Using MACS Dead Cell Removal Kit, even early apoptotic cells with an intact cellular membrane are removed.

Options for the Analysis of Dead Cell Removal

Removal of dead cells can be analyzed by microscopy with membrane exclusion dyes like Trypan Blue or by flow cytometric analysis using Propidium Iodide.[1]

Example for elimination of dead cells from tissue culture using Dead Cell Removal Kit

Dead cells were eliminated from cultured Jurkat cells by labeling of cells with Dead Cell Removal MicroBeads and separation over an LS Column in the magnetic field of a MidiMACS Separator. Dead Cells can be fluorescently stained with Propidium Iodide.



Forward Scatter versus staining with Propidium Iodide.

References

1. Coder, DM (1997) Assessment of Cell Viability. In: Robinson, JP *et al*. (eds.) Current Protocols in Cytometry, p. 9.2.1-9.2.14, John Wiley & Sons Inc., New York

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. MILTENYI BIOTEC GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the Technical Specifications of the products. MILTENYI BIOTEC GmbH's liability is limited to either replacement of the products or refund of the purchase price. MILTENYI BIOTEC GmbH is not liable for any property damage, personal injury or economic loss caused by the product. Purchase of the products does not include a license to perform any patented application.

140001193.04