Miltenyi Biotec

Endothelial Cell Isolation Kit

rat

Order no. 130-109-679

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 Non-Endothelial Cell Depletion Cocktail, rat**
	1 mL Endothelial Cell Isolation Cocktail, rat
Capacity	For up to 5×10^9 total cells, up to 50 separations.
Product format	All components 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 MACS® Separation

The isolation of endothelial cells is performed in a two-step procedure. First, the non-endothelial cells are magnetically labeled with the Non-Endothelial Cell Depletion Cocktail. The labeled cells are subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator.

In the second step, the endothelial cells are labeled with the Endothelial Cell Isolation Cocktail and isolated by positive selection from the non-endothelial cell–depleted fraction by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator.

After removing the column from the magnetic field, the magnetically retained endothelial cells can be eluted as the positively selected cell fraction.

Rat tissue: Depletion of non-endothelial cells

1. Magnetic labeling of non-endothelial cells with Non-Endothelial Cell Depletion Cocktail.
2. Magnetic separation using an LD Column.

Pre-enriched endothelial cells (flow-through fraction): Positive selection of endothelial cells

1. Magnetic labeling of endothelial cells with Endothelial Cell Isolation Cocktail.
2. Magnetic separation using an MS Column.

Enriched endothelial cells

1.2 Background information

Endothelial cells form the inner layer of blood vessels and play a key role in vascular development, homeostasis and remodeling. The Endothelial Cell Isolation Kit, rat has been designed for the enrichment of endothelial cells from dissociated adult rat hearts, muscle, fat, and neonatal brain.

For optimal use, the Endothelial Cell Isolation Kit should be used in combination with the appropriate dissociation kit. Please refer to section 1.4 for more information.

1.3 Applications

- Enrichment of endothelial cells from adult rat hearts, muscle, fat, and neonatal brain.
- Culture and expansion or direct use of enriched endothelial cells for biochemical, physiological, and pharmacological studies.

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2 and 0.5% bovine serum albumin (BSA). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.
  Always use freshly prepared buffer. Do **not use** autoMACS® Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

140-004-997.01

- MACS® Columns and MACS Separators: For optimal purity and recovery the use of an LD Column for depletion of non-endothelial cells is strongly recommended. For the subsequent positive selection of endothelial cells the use of an MS Column is strongly recommended. Please use buffers for separation as indicated in this section.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	5×10^7	1×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS, VarioMACS, SuperMACS II

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

- (Optional) Neonatal Heart Dissociation Kit (# 130-098-373) for the generation of single-cell suspension from adult rat hearts.
- (Optional) Skeletal Muscle Dissociation Kit (# 130-098-305) for the generation of single-cell suspension from adult rat muscle tissue.
- (Optional) Adipose Tissue Dissociation Kit (# 130-105-808) for the generation of single-cell suspension from adult rat fat tissue.
- (Optional) Neural Tissue Dissociation Kit (P) (# 130-092-628) for the generation of single-cell suspension from neonatal rat brain tissue.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) and gentleMACS C Tubes (# 130-093-237, # 130-096-334).
- (Optional) Debris Removal Solution (# 130-109-398) to remove debris after tissue dissociation, e.g., of adult rat hearts.
- (Optional) MACS SmartStrainers (70 µm) (# 130-098-462) or Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- (Optional) Human Fibronectin (Fragment) (# 130-109-393) for counting of cell culture dishes prior to plating of enriched endothelial cells.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Labeling Check Reagent-VioBlue® (# 130-095-087), CD31-VioBright™ FITC (# 130-105-880), and CD90.1-PE (# 130-102-636). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

2. Protocol

2.1 Sample preparation

For preparation of single-cell suspensions use the appropriate dissociation kit in combination with the gentleMACS Dissociators.

For efficient plating and culture of isolated endothelial cells it is strongly recommended to use fibronectin fragment–coated cell culture dishes. Coat cell culture dishes with 3–5 µg/cm² Human Fibronectin (Fragment) at least for 2 hours at 37 °C. Before use aspirate the coating solution and add the cell suspension immediately.

2.2 Magnetic labeling of non-endothelial cells

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for:

up to 10^7 total cells, when working with muscle, fat, or neonatal brain tissue,

up to 10^8 total cells, when working with adult heart tissue.

When working with fewer than 10^7 or 10^8 cells, respectively, 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 or 2×10^8 total cells, respectively, 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 70 µm nylon mesh (Pre-Separation Filters (70 µm), # 130-095-823) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer.
4. Add 20 µL of Non-Endothelial Cell Depletion Cocktail.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Adjust volume to 500 µL using buffer. Do not centrifuge.
7. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-endothelial cells

▲ Choose an LD Column and MACS Separator. For details refer to table in section 1.4.

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

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with

140-004-997.01

2×1 mL of buffer. Collect total effluent; this is the unlabeled pre-enriched endothelial cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 3 mL of buffer onto the column. Immediately flush out the magnetically labeled non-endothelial cells by firmly pushing the plunger into the column.
6. Proceed to 2.4 for the labeling of endothelial cells.

2.4 Magnetic labeling of endothelial cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^7 total cells when muscle, fat, or neonatal brain tissue has been used as starting material, and 10^8 when adult heart tissue has been used as starting material. For higher initial cell numbers, scale up all volumes accordingly.

1. Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer.
3. Add 20 µL of Endothelial Cell Isolation Cocktail.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. Adjust volume to 500 µL using buffer. Do not centrifuge.
6. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of endothelial cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS® Separator. For details refer to MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

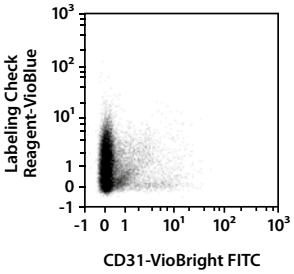
   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

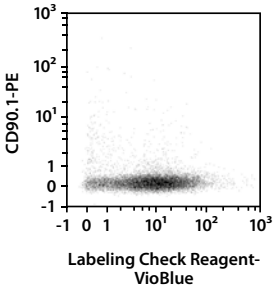
3. Example of a separation using the Endothelial Cell Isolation Kit

Endothelial cells were isolated from adult CD-1® rat hearts by using the Endothelial Cell Isolation Kit, one LD Column with MidiMACS™ Separator, and one MS Column with MiniMACS™ Separator. The cells were fluorescently stained with Labeling Check Reagent-VioBlue® and 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 fluorescence.

Before separation



Enriched endothelial cells



Refer to **www.miltenyibiotec.com** for all data sheets and protocols.