Miltenyi Biotec

Neonatal Cardiac Endothelial Cell Isolation Kit

rat

Order no. 130-106-768

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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-Cardiac Endothelial Cell Depletion Cocktail, rat** **1 mL Cardiac Endothelial Cell Isolation Cocktail, rat**
Capacity	For 5×10^8 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 cardiac endothelial cells is performed in a two-step procedure. First, the non-endothelial cells are magnetically labeled with Non-Cardiac 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 cardiac endothelial cells are labeled with Cardiac 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 neonatal cardiac endothelial cells can be eluted as the positively selected cell fraction.

Neonatal rat hearts (P0–P3): Depletion of non-endothelial cells

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

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

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

Neonatal cardiac endothelial cells

1.2 Background information

Besides cardiomyocytes and fibroblasts, endothelial cells represent the third most frequent cell population in the neonatal rat heart. Endothelial cells form the inner layer of blood vessels and play a key role in vascular development, homeostasis, and remodeling. The Neonatal Cardiac Endothelial Cell Isolation Kit, rat has been designed for enrichment of cardiac endothelial cells from dissociated neonatal rat hearts. The isolation of cardiac endothelial cells has successfully been tested using neonatal rat hearts from postnatal day 0 to day 3 (P0–P3). For optimal use, the Neonatal Cardiac Endothelial Cell Isolation Kit should be used in combination with the Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373).

1.3 Applications

- Enrichment of cardiac endothelial cells from neonatal rat hearts (P0-P3).
- Culture and expansion or direct use of enriched cardiac 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.

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140-004-793.02

▲ **Note:** Addition of DNase (230 U/mL) to the buffer is optional, but may improve plating of endothelial cells after cell separation.

▲ **Note:** BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing $Ca2^+$ or $Mg2^+$ are not recommended for use.

- MACS Columns and MACS Separators: For optimal purity and recovery the use of an LD Column for depletion of non-cardiac endothelial cells is strongly recommended. For the subsequent positive selection of neonatal cardiac 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	10^7	2×10^7	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.

- Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373) for the generation of single-cell suspension from neonatal heart tissue.
- Red Blood Cell Lysis Solution (10×) (# 130-094-183).
- 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) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD90.1-PE (# 130-102-636) or CD31-APC (# 130-105-937). 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.
- (Optional) MACS SmartStrainers (70 µm) (# 130-098-462) or Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.

2. Protocol

2.1 Sample preparation

For preparation of single-cell suspensions from neonatal hearts use the Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373) in combination with the gentleMACS Dissociator (# 130-092-235), the gentleMACS Octo Dissociator (# 130-095-937), or the gentleMACS Octo Dissociator with Heaters (# 130-096-427).

For optimal performance a red blood cell lysis before magnetic labeling and separation by using Red Blood Cell Lysis Solution (10×) (# 130-094-183) has to be performed. For details, especially optimal incubation time, refer to the Neonatal Heart Dissociation Kit, mouse and rat data sheet.

For efficient plating and culture of isolated cardiac endothelial cells it is strongly recommended to use fibronectin-coated cell culture dishes. Coat cell culture dishes with fibronectin (50 µg/mL) at least for 2 hours in the incubator. 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 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 70 µm nylon mesh (MACS SmartStrainers (70 µm) (# 130-098-462) or 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. Working on ice may require increased incubation times. 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 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of Non-Cardiac Endothelial Cell Depletion Cocktail per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
6. Adjust volume to 500 µL using buffer for 10^7 total cells. Do not centrifuge.
7. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: Depletion of non-endothelial cells

▲ Choose an LD Column and Separator. For details refer to the 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 the 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 2×1 mL of buffer. Collect total effluent; this is the unlabeled pre-enriched endothelial cell fraction.
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 neonatal cardiac endothelial cells.

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2.4 Magnetic labeling of cardiac endothelial cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^7 total cells. For higher initial cell numbers, scale up all volumes accordingly.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer.
3. Add 20 µL of Cardiac Endothelial Cell Isolation Cocktail.
4. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
5. Wash cells by adding 1–2 mL of buffer per 10^7 initial cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^7 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
7. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of neonatal cardiac endothelial cells

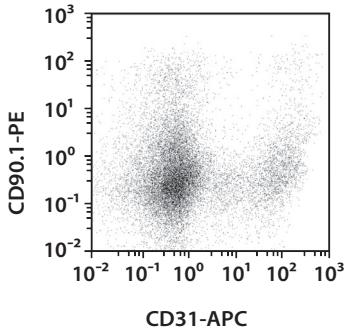
Positive selection with MS Columns

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 only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable 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.

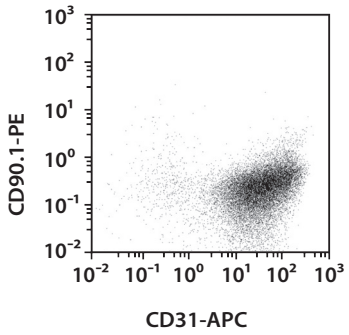
3. Example of a separation using the Neonatal Cardiac Endothelial Cell Isolation Kit

Cardiac endothelial cells were isolated from P1 Wistar rat hearts by using the Neonatal Cardiac Endothelial Cell Isolation Kit, one LD Column with MidiMACS™ Separator, and one MS Column with MiniMACS™ Separator. The cells were fluorescently stained with CD90.1-PE (# 130-102-636) and CD31-APC (# 130-105-937) 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 cardiac endothelial cells



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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