Miltenyi Biotec

Neonatal Cardiac Fibroblast Isolation Kit
rat

Order no. 130-105-422

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-Cardiac Fibroblast Depletion Cocktail, rat**
	1 mL Cardiac Fibroblast 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 fibroblasts is performed in a two-step procedure. First, non-fibroblasts are magnetically labeled with the Non-Cardiac Fibroblast 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, cardiac fibroblasts are labeled with the Cardiac Fibroblast Isolation Cocktail and isolated by positive selection from the pre-enriched non-fibroblast–depleted cell 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 fibroblasts can be eluted as the positively selected cell fraction.

Neonatal rat hearts (P0–3): Depletion of non-fibroblasts

1. Magnetic labeling of non-fibroblasts with Non-Cardiac Fibroblast Depletion Cocktail.
2. Magnetic separation using an LS Column or an autoMACS Column (program "Depletes").

Pre-enriched cardiac fibroblasts (flow-through fraction): Positive selection of cardiac fibroblasts

1. Magnetic labeling of fibroblasts with Cardiac Fibroblast Isolation Cocktail.
2. Magnetic separation using an LS Column or an autoMACS Column (program "Possels").

Neonatal cardiac fibroblasts

1.2 Background information

Cardiac fibroblasts are one of the most frequent cell types within the heart and play a critical role (i) in heart development, (ii) in maintaining normal cardiac function, as well as (iii) in cardiac remodeling during pathological conditions such as myocardial infarction. Moreover, cardiac fibroblasts are believed to be an ideal target population to be reprogrammed into cardiomyocyte-like cells for regenerative purposes.

The Neonatal Cardiac Fibroblast Isolation Kit, rat has been designed for the enrichment of cardiac fibroblasts from dissociated neonatal rat hearts.

The isolation of cardiac fibroblasts has been tested using neonatal rat hearts from postnatal day 0 to day 3 (P0–3). For optimal results, the Neonatal Cardiac Fibroblast 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 fibroblasts from neonatal (P0–3) rat hearts.
- Culture and expansion or direct use of enriched cardiac fibroblasts for biochemical, physiological, and pharmacological studies.

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1.4 Reagent and instrument requirements

- PEB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). 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.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as rat serum albumin, rat serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: For optimal purity and recovery the use of LS Columns is strongly recommended. Separation can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	10^7	2×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	10^7	2×10^7	autoMACS Pro, autoMACS

  ▲ **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.
- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- MACS SmartStrainers (70 µm) (# 130-098-462) or Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) Labeling Check Reagent-PE (# 130-095-228). 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 from neonatal hearts use the Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373) in combination with the gentleMACS Dissociators.

For efficient plating and culture of isolated cardiac fibroblast it is strongly recommended to use gelatin-coated cell culture dishes. Coat cell culture dishes with 0.1% gelatin for at least 2 hours in the incubator. Before use aspirate the gelatin solution and add the cell suspension immediately.

2.2 Magnetic labeling of non-fibroblasts

▲ For optimal performance it is recommended to perform a red blood cell lysis before magnetic labeling and separation by using Red Blood Cell Lysis Solution (10×) (# 130-094-183). For details, e.g., regarding optimal incubation time, refer to the Neonatal Heart Dissociation Kit, mouse and rat data sheet.

▲ 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 a 70 µm nylon mesh (MACS SmartStrainer (70 µm) # 130-098-373; or Pre-Separation Filters, 70 µm, # 130-098-462) 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 PEB buffer per 10^7 total cells.
4. Add 20 µL of the Non-Cardiac Fibroblast Depletion Cocktail per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Adjust volume to 500 µL using PEB buffer for up to 10^7 total cells. Do not centrifuge.
7. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-fibroblasts

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

Magnetic separation with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to the respective LS Column data sheet.
2. Prepare column by rinsing with 3 mL of PEB 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 flow-through; this is the unlabeled cell fraction representing the pre-enriched cardiac fibroblasts. 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 1 mL of buffer onto the column. Immediately flush out the magnetically labeled non-cardiac fibroblasts by firmly pushing the plunger into the column.
6. Proceed to 2.4 for the labeling of cardiac fibroblasts.

Depletion with the autoMACS® Pro Separator or the autoMACS® Separator

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥10 °C. Do not use the autoMACS Running Buffer for enrichment of fibroblasts. Use the buffer composition as described in section 1.4.

Magnetic separation with the autoMACS® Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack. This fraction represents the pre-enriched cardiac fibroblasts.
4. Proceed to 2.4 for the labeling of cardiac fibroblasts.

Magnetic separation with the autoMACS® Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction from outlet port neg1. This fraction represents the pre-enriched cardiac fibroblasts.
4. Proceed to 2.4 for the labeling of cardiac fibroblasts.

2.4 Magnetic labeling of cardiac fibroblasts

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 1×10^7 total cells. 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 PEB buffer.
3. Add 20 µL of Cardiac Fibroblast Isolation Cocktail.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. Adjust volume to 500 µL using PEB buffer (for up to 10^7 initial cells). Do not centrifuge.
6. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of cardiac fibroblasts

Positive selection with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to LS Column data sheet.
2. Prepare column by rinsing with 3 mL of PEB 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 flow-through; this is the unlabeled cell fraction representing the non-fibroblasts. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled cardiac fibroblasts by firmly pushing the plunger into the column.

Positive selection with the autoMACS® Pro Separator or the autoMACS® Separator

Magnetic separation with the autoMACS® Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Positive selection: Possel
   Collect positive fraction in row C of the tube rack. This is the enriched cardiac fibroblast cell fraction.

Magnetic separation with the autoMACS® Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

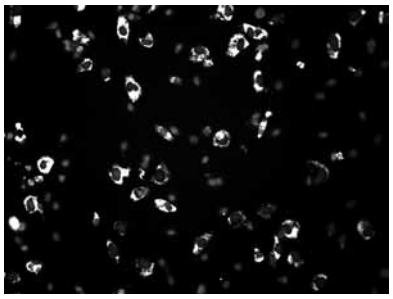
   Positive selection: Possels
   Collect positive fraction from outlet port pos1. This is the enriched cardiac fibroblast cell fraction.

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

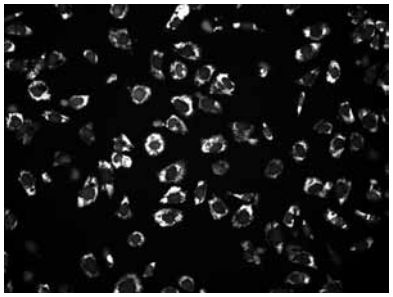
Rat neonatal hearts were dissociated using the Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373) in combination with the gentleMACS Octo Dissociator with Heaters. Subsequently, neonatal cardiac fibroblasts were isolated using the Neonatal Cardiac Fibroblast Isolation Kit, rat and cultured in DMEM medium with 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin for 1 day.

Immunofluorescence pictures show labeling of cardiac fibroblasts with an anti-Prolyl 4-hydroxylase β antibody before (A) and after (B) magnetic enrichment using the Neonatal Cardiac Fibroblast Isolation Kit, rat. Nuclei are visualized by DAPI staining (dark grey).

A)



B)



All protocols and data sheets are available at **www.miltenyibiotec.com**.

Warranty

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