Miltenyi Biotec

Neonatal Cardiac Fibroblast Isolation Kit
mouse

Order no. 130-101-372

Contents

Neonatal mouse hearts (P0–P3): Depletion of non-fibroblasts

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

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 MS Column or an autoMACS Column (program "Possel").

Neonatal cardiac fibroblasts

1. Description

Components	**1 mL Non-Cardiac Fibroblast Depletion Cocktail, mouse** **1 mL Cardiac Fibroblast Isolation Cocktail, mouse**
Capacity	For 5×10^8 total cells, up to 100 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 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 magnetic 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.

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.
The Neonatal Cardiac Fibroblast Isolation Kit, mouse has been designed for the enrichment of cardiac fibroblasts from dissociated neonatal mouse hearts.
The isolation of cardiac fibroblasts has been successfully tested using neonatal mouse hearts from postnatal day 0 to day 3 (P0–P3). 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-P3) mouse hearts.
- Culture and expansion or direct use of enriched cardiac fibroblasts for biochemical, physiological and pharmacological studies.

1.4 Reagent and instrument requirements

- 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.

  ▲ **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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-004-231.02

- MACS Columns and MACS Separators: For optimal purity and recovery the use of an MS Column 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
Depletion			
MS	5×10^6	10^7	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
autoMACS	5×10^6	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.

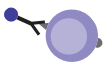
- Neonatal Heart Dissociation Kit (# 130-098-373) for the generation of single-cell suspension from neonatal heart tissue.
- gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937) and gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- Pre-Separation Filters, 70 µm (# 130-095-823) to remove cell clumps.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD90.2-APC or CD31-PE. 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 without fixation.

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) or the gentleMACS Octo Dissociator (# 130-095-937).

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

▲ 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 5×10^6 total cells. When working with fewer than 5×10^6 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 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 2–8 °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 90 µL of buffer per 5×10^6 total cells.
4. Add 10 µL of Non-Cardiac Fibroblast Depletion Cocktail per 5×10^6 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Adjust volume to 500 µL using 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

▲ Choose an appropriate MACS 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.

Magnetic separation with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to the respective 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, representing the pre-enriched cardiac fibroblasts.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only 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-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.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

140-004-231.02

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: "Deplete"
   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: Deplete
   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 5×10^6 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 90 µL of buffer.
3. Add 10 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 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 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, representing non-fibroblasts.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through, representing non-fibroblasts, 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 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.

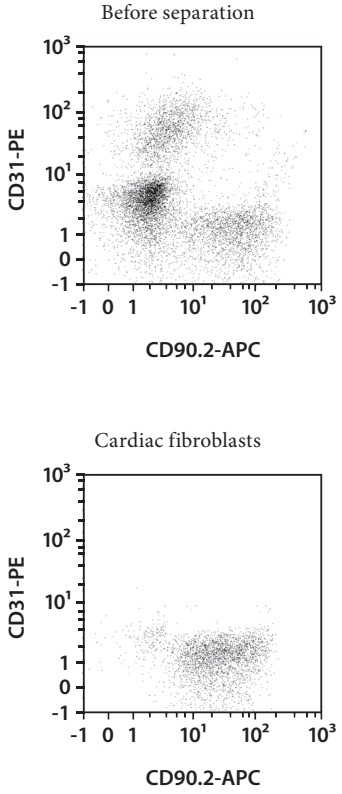
Magnetic separation with the autoMACS® Separator

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: Possel
   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

Cardiac fibroblasts were isolated from P2 CD-1® mouse hearts by using the Neonatal Cardiac Fibroblast Isolation Kit, two MS Columns, and a MiniMACS™ Separator. The cells were fluorescently stained with CD90.2-APC and CD31-PE 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.



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.

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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, gentleMACS, MACS, MACSQuant, the Miltenyi Biotec logo, MiniMACS, OctoMACS, SuperMACS, and VarioMACS are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.