Miltenyi Biotec

Neonatal Cardiomyocyte Isolation Kit

mouse

Order no. 130-100-825

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 Neonatal Cardiomyocyte Isolation Cocktail, mouse.
Capacity	For 5×10^8 total cells, up to 100 separations.
Product format	Cocktail is 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

Using the Neonatal Cardiomyocyte Isolation Kit, mouse cardiomyocytes are isolated by depletion of non-target cells. Non-target cells are directly magnetically labeled with a cocktail of monoclonal antibodies conjugated with MACS® MicroBeads. The magnetically labeled non-target cells are depleted by retaining them within a MACS Column in the magnetic field of a MACS Separator, while the unlabeled cardiomyocytes pass through the column.

1.2 Background information

One of the most used experimental models in cardiac research is the isolation of vital cardiac cells from neonatal hearts. Especially the isolation and subsequent culture of cardiomyocytes for biochemical, physiological, pharmacological and morphological studies have a high impact in the field of cardiovascular research.
The Neonatal Cardiomyocyte Isolation Kit, mouse has been designed for the enrichment of untouched cardiomyocytes from dissociated neonatal mouse hearts.
The isolation of cardiomyocytes has been successfully tested using neonatal mouse hearts from postnatal day 0 to day 3 (P0–P3). For optimal results, the Neonatal Cardiomyocyte Isolation Kit should be used in combination with the Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373).

1.3 Applications

- Enrichment of untouched cardiomyocytes from neonatal mouse hearts (P0–P3).
- Culture or direct use of enriched cardiomyocytes for biochemical, physiological, pharmacological, and morphological 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.
  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 mouse serum albumin, mouse 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 an MS Column is strongly recommended. Depletion can also be performed by using the autoMACS Pro 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

  ▲ **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), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Dissociator with Heaters (# 130-096-427)

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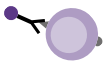
- gentleMACS C Tubes (# 130-093-237, # 130-096-334).
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- (Optional) Inside Stain Kit (# 130-090-477) to detect intracellular markers, such as α-actinin.
- (Optional) Propidium Iodide 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.

For efficient plating and culture of isolated cardiomyocytes it is strongly recommended to use fibronectin-coated cell culture dishes. Coat cell culture dishes with fibronectin (20–100 µg/mL) for at least 2 hours in the incubator. Before use aspirate the fibronectin solution and add the cell suspension immediately.



2.2 Magnetic labeling

▲ 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.

▲ If cells have to be cultured separation can also be performed in standard culture media instead of buffer.

▲ 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 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 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 90 µL of buffer per 5×10^6 total cells.
4. Add 10 µL of Neonatal Cardiomyocyte Isolation Cocktail per 5×10^6 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 up to 10^7 total cells. Do not centrifuge.
7. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS 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 MACS 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 enriched neonatal cardiomyocytes.
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-cardiomyocytes by firmly pushing the plunger into the column.

Magnetic separation with the autoMACS® Pro Separator

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

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

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 one of the following programs:

   Depletion: Deplete
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched neonatal cardiomyocytes.
5. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non–cardiomyocytes.

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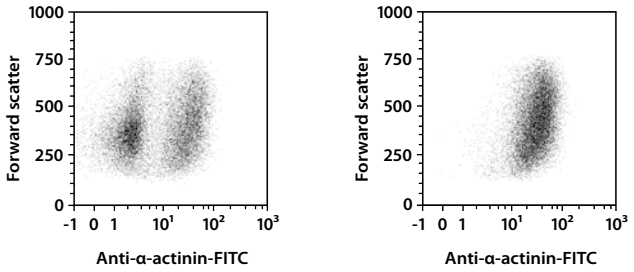
3. Example of a separation using the Neonatal Cardiomyocyte Isolation Kit

Neonatal cardiomyocytes were isolated from P2 CD-1® mouse hearts using the Neonatal Heart Dissociation Kit, mouse and rat, the Neonatal Cardiomyocyte Isolation Kit, mouse, an MS Column, and a MiniMACS™ Separator. Subsequently, cells were fixed and stained using the Inside Stain Kit (# 130-090-477) and a FITC-conjugated antibody specific for cardiomyocytes (anti-α-actinin). Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris were excluded from the analysis based on scatter signals.

Before separation **After separation**



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

Warranty

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