Miltenyi Biotec

Neonatal Cardiomyocyte Isolation Kit

rat

Order no. 130-105-420

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, rat** **1 mL Anti-Red Blood Cell MicroBeads, rat**
Capacity	For 10^9 total cells, up to 50 separations.
Product format	Neonatal Cardiomyocyte Isolation Cocktail is supplied in buffer containing stabilizer and 0.05% sodium azide. Anti-Red Blood Cell MicroBeads are supplied in buffer containing stabilizer.
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

Cardiomyocytes are isolated by depletion of non-target cells. First, non-target cells are directly magnetically labeled with a cocktail of monoclonal antibodies conjugated with MACS® MicroBeads. Then, the cell suspension is loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled non-target cells are retained within the column. The unlabeled cardiomyocytes run through.

1.2 Background information

One of the most used experimental model 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 has a high impact in the field of cardiovascular research.

The Neonatal Cardiomyocyte Isolation Kit, rat has been designed for the enrichment of untouched cardiomyocytes from dissociated neonatal rat hearts.

The isolation of cardiomyocytes has been tested using neonatal rat hearts from postnatal day 0 to day 3 (P0–3). 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 rat hearts (P0–3).
- Culture or direct use of enriched cardiomyocytes for biochemical, physiological, pharmacological, and morphological studies.

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 an LD Column is strongly recommended. Depletion can also be performed by using the MultiMACS™ Cell24 Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LD	10^7	2×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Multi-24	10^7	2×10^7	MultiMACS Cell24

▲ **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.

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140-004-650.03

- 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.
- (Optional) Human Fibronectin (Fragment) (# 130-109-393)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., α-Actinin (Sarcomeric) Antibody-FITC, Cardiac Troponin T Antibody-FITC, Myosin Heavy Chain Antibody-VioBlue®, MLC2a Antibody-PE, or MLC2v Antibody-APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Inside Stain Kit (# 130-090-477) to detect intracellular cardiomyocyte-specific proteins, such as alpha-actinin, troponin T, myosin heavy chain, MLC2a, or MLC2v.

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 details refer to www.gentlemacs.com/protocols.

For efficient plating and culture of isolated cardiomyocytes it is strongly recommended to use fibronectin-coated cell culture dishes. Coat cell culture dishes with 3–5 µg/cm² Human Fibronectin (Fragment) for at least two hours at 37 °C. Before use, aspirate the supernatant completely and add the cell suspension immediately.

2.2 Magnetic labeling

▲ 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 2×10^7 total cells. When working with fewer than 2×10^7 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 4×10^7 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 SmartStrainer (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. 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 60 µL of buffer per 2×10^7 total cells.
4. Add 20 µL of Neonatal Cardiomyocyte Isolation Cocktail per 2×10^7 total cells.
5. Add 20 µL of Anti-Red Blood Cell MicroBeads per 2×10^7 total cells.
6. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
7. Adjust volume to 500 µL using buffer for up to 2×10^7 total cells. Do not centrifuge.
8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an LD Column and an appropriate 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.

Depletion with LD Columns

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

Magnetic separation with the MultiMACS™ Cell24 Separator

Refer to the the MultiMACS™ Cell Separator user manual for instructions on how to use the MultiMACS Cell24 Separator.

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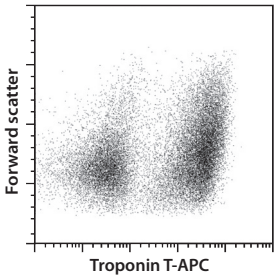
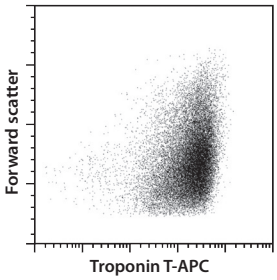
3. Example of a separation using the Neonatal Cardiomyocyte Isolation Kit

Neonatal cardiomyocytes were isolated from P2 Wistar rat hearts using the Neonatal Heart Dissociation Kit, mouse and rat, the Neonatal Cardiomyocytes Isolation Kit, rat, an LD Column, and a MidiMACS™ Separator. Subsequently, cells were fixed and stained using the Inside Stain Kit and Cardiac Troponin T Antibody-FITC specific for cardiomyocytes. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris was excluded from the analysis based on scatter signals.

Before separation



Neonatal cardiomyocytes



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.

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