Miltenyi Biotec

PSC-Derived Cardiomyocyte Isolation Kit human

Order no. 130-110-188

Contents

1. Description

This product is for research use only.

Components	**1 mL Non-Cardiomyocyte Depletion Cocktail, human:** Cocktail of biotinylated antibodies.
	1 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).
	1 mL Cardiomyocyte Enrichment Cocktail, human
Capacity	For 2.5×10^8 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 human pluripotent stem cell (PSC)-derived cardiomyocytes is performed in a two-step procedure. First, the PSC-derived non-cardiomyocytes are magnetically labeled with the Non-Cardiomyocyte Depletion Cocktail and Anti-Biotin MicroBeads. 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 PSC-derived cardiomyocytes are magnetically labeled with Cardiomyocyte Enrichment Cocktail and isolated by positive selection from the pre-enriched PSC-derived cardiomyocytes 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 PSC-derived cardiomyocytes can be eluted as the positively selected cell fraction.

Pluripotent stem cell (PSC)-derived cardiomyocytes: Depletion of PSC-derived non-cardiomyocytes

1. Indirect magnetic labeling of non-cardiomyocytes with Non-Cardiomyocyte Depletion Cocktail and Anti-Biotin MicroBeads.
2. Magnetic separation using an LS Column or an autoMACS Column (program "Deplete").

Pre-enriched PSC-derived cardiomyocytes (flow-through fraction): Positive selection of PSC-derived cardiomyocytes

1. Direct magnetic labeling of PSC-derived cardiomyocytes with the Cardiomyocyte Enrichment Cocktail.
2. Magnetic separation using an LS Column or an autoMACS Column (program "Possel").

PSC-derived cardiomyocytes

1.2 Background information

Pure and well-characterized cardiomyocytes derived from human pluripotent stem cells (PSCs) are of high interest for cardiovascular disease modeling, drug safety studies, and development of cell replacement strategies. Although several protocols for cardiac differentiation of PSCs have been developed, there are still major limitations, including clone-to-clone and experiment-to-experiment variation in differentiation efficacy. To circumvent these limitations the PSC-Derived Cardiomycoyte Isolation Kit, human has been developed. It enables the purification of PSC-derived cardiomyocytes to high purities from various differentiation efficiencies, stem cell clones, differentiation time points, and differentiation protocols. For optimal use, the PSC-Derived Cardiomyocyte Isolation Kit, human should be used in combination with the Multi Tissue Dissociation Kit 3 for harvesting of cardiomyocytes prior to magnetic cell separation.

1.3 Applications

- Enrichment of human PSC-derived cardiomyocytes.
- Cultivation, expansion or direct use of pure human PSC-derived cardiomyocyte for biochemical, physiological, and pharmacological studies.

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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 human serum albumin, human 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 for depletion of non-cardiomyocytes as well as for the subsequent positive selection of PSC-derived cardiomyocytes is strongly recommended. Positive selection and depletion can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion or positive selection			
LS	5×10^6	5×10^6	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	10^7	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.

- Multi Tissue Dissociation Kit 3 for the generation of a single-cell suspension from PSC-derived cardiac monolayer culture (# 130-110-204).
- MACS SmartStrainers (70 µm) (# 130-098-462) or Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g. Anti-Cardiac Troponin T-FITC (# 130-106-687), Anti-α-Actinin (Sarcomeric)-FITC (# 130-106-936), Anti-Myosin Heavy Chain-APC (# 130-106-215), Anti-MLC2a-APC (# 130-106-143), or Anti-MLC2v-PE (# 130-106-133). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) If PSC-derived cardiomyocytes are further cultivated after separation, supplementation of culture medium with StemMACS Thiazovivin (# 130-104-461) is recommended for the first day of cultivation.
- (Optional) Human Fibronectin (Fragment) (# 130-109-393) as coating matrix.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

2. Protocol

2.1 Sample preparation

For the generation of a single-cell suspension from cardiac monolayer differentiations the use of the Multi Tissue Dissociation Kit 3 is strongly recommended.

2.2 Magnetic labeling of PSC-derived non-cardiomyocytes

▲ 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 200×g for 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 5×10^6 total cells.
4. Add 20 µL of Non-Cardiomyocyte Depletion Cocktail per 5×10^6 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1 mL of buffer per 5×10^6 cells and centrifuge at 200×g for 5 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 80 µL of buffer per 5×10^6 total cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 5×10^6 total cells.
9. Mix well and incubate for additional 10 minutes in the refrigerator (2–8 °C).
10. Adjust volume to 500 µL using buffer for up to 10^7 total cells. Do not centrifuge.
11. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation: Depletion of PSC-derived non-cardiomyocytes

▲ Choose an LS 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.

Magnetic separation with LS Columns

1. Place 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 buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched PSC-derived cardiomyocytes.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched PSC-derived cardiomyocytes, and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

   ▲ **Note:** Purity of the enriched PSC-derived cardiomyocyte population might be sufficient without the additional enrichment step (2.4). Proceed directly to plating and culturing of PSC-derived cardiomyocytes (2.6).
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-cardiomyocytes by firmly pushing the plunger into the column.
6. Proceed to magnetic labeling (2.4).

Depletion 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 the following program:

   Depletion: Deplete
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched PSC-derived cardiomyocytes.
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-PSC-derived cardiomyocytes.
5. Proceed to magnetic labeling (2.4).

2.4 Magnetic labeling of PSC-derived cardiomyocytes

▲ 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 200×g for 5 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer.
3. Add 20 µL of Cardiomyocyte Enrichment Cocktail.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. Adjust volume to 500 µL using buffer for up to 10^7 total cells. Do not centrifuge.
6. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of PSC-derived cardiomyocytes

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 buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as 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 cells by firmly pushing the plunger into the column.
7. For subsequent plating and culturing proceed to 2.6

Positive selection 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 PSC-derived cardiomyocyte fraction.
4. For subsequent plating and culturing proceed to 2.6

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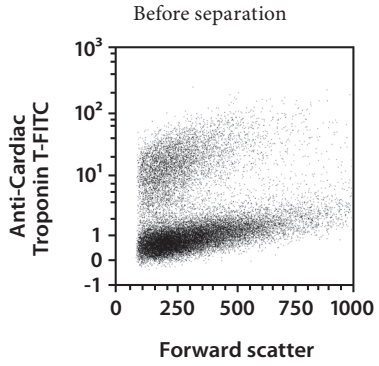
2.6 Plating and culturing of PSC-derived cardiomyocytes

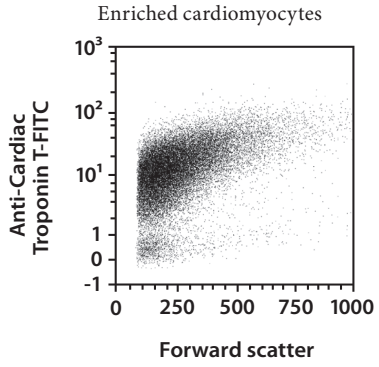
For subsequent cardiomyocyte plating and culture cell culture plates need to be pre-coated with either matrigel or Human Fibronectin (Fragment).

1. Centrifuge the enriched PSC-derived cardiomyocyte suspension at 200×g for 5 minutes. Aspirate supernatant completely.
2. Resuspend an appropriate amount of enriched PSC-derived cardiomyocytes in an adequate medium supplemented with StemMACS Thiazovivin (2 µM; # 130-104-461).
3. Add the cell suspension to the coated dishes. A plating density of 300.000 cells/cm² is recommended.

3. Example of a separation using the PSC-Derived Cardiomyocyte Isolation Kit

Cardiomyocytes were isolated from a day 14 differentiation by using the Multi Tissue Dissociation Kit 3, the PSC-Derived Cardiomyocyte Isolation Kit, and two LS Columns with a MidiMACS™ Separator. The cells were fluorescently stained with Anti-Cardiac Troponin T-FITC 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.





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