Miltenyi Biotec

StemMACS™ CardioDiff Kit XF
human

Order no. 130-125-289

Contents

1. Description

This product is for research use only.

Components	**2×500 mL StemMACS CardioDiff Basal Medium XF, human** **1×5 mL StemMACS CardioDiff Mesoderm Induction Supplement XF (20×), human** **2×10 mL StemMACS CardioDiff Cardiac Cultivation Supplement XF (50×), human** **1×5 mL StemMACS CardioDiff Cardiac Induction Supplement XF (20×), human**
Specifications	Prepared Mesoderm Induction Medium Osmolality: 270–320 mOsmol/kg pH: 7.1–7.6 Prepared Cardiac Cultivation Medium Osmolality: 260–310 mOsmol/kg pH: 7.1–7.6 Prepared Cardiac Induction Medium Osmolality: 260–310 mOsmol/kg pH: 7.1–7.6
Capacity	For 48 assays. One assay corresponds to one well of a 12-well plate.
Storage	Upon arrival, store StemMACS CardioDiff Basal Medium XF, human protected from light at +2 to +8 °C. Store StemMACS CardioDiff Kit XF Supplements protected from light at –20 °C. The expiration date is indicated on the label. Avoid repeated freeze-thaw-cycles. Once prepared, keep the complete media at +2 to +8 °C and use within 4 weeks.

1.1 Background information

Directed differentiation of specific lineages from human pluripotent stem cells (hPSCs) is a major tool for developmental or disease models[1], drug screening platforms[2] and cellular therapies[3]. StemMACS CardioDiff Kit XF, human is a complete, ready-to-use, and xeno-free cell culture system for the efficient and fast differentiation of hPSCs into cardiomyocytes. This product is without phenol red. The kit is composed of three media that progressively restrict the cellular fate and promote the differentiation into cardiomyocytes in just 8 days of culture. Depending on the cellular line, first contracting cardiomyocytes can be observed already after 6 days of culture. PSCs-derived cardiomyocyte can be further expanded in StemMACS CardioDiff Cardiac Cultivation Medium XF, human and cultivated for more than 30 days.

1.2 Applications

- Directed differentiation of cardiomyocytes from hPSCs lines.

1.3 Reagent and instrument requirements

- Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}
- 0.05% Trypsin/EDTA
- Soybean Trypsin Inhibitor (0.5 mg/mL)
- Corning® Matrigel® hESC-qualified Matrix
- DMEM/F12 with L-Glutamine, without HEPES
- (Optional) Multi Tissue Dissociation Kit 3 (# 130-110-204)

2. Protocol

2.1 Protocol overview

Day	Action	Complete medium
0	Plate cells	Mesoderm Induction Medium*
1	Change media	Cardiac Cultivation Medium*
2	Change media	Cardiac Induction Medium*
3	Change media	Cardiac Cultivation Medium*
4	Change media	
5	Change media	
6	Change media	
7	Change media	
8	Change media	

* For media preparation refer to table 1.

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2.2 Preparation of complete media

▲ Kit components should not be substituted or mixed with those from other kits or lots.

▲ Avoid repeated freeze-thaw cycles.

Complete media	Component	Amount [mL]
Mesoderm Induction Medium	StemMACS CardioDiff Basal Medium XF, human	95
	StemMACS CardioDiff Mesoderm Induction Supplement XF (20×), human	5
Cardiac Cultivation Medium	StemMACS CardioDiff Basal Medium XF, human	800
	StemMACS CardioDiff Cardiac Cultivation Supplement XF (50×), human	16
Cardiac Induction Medium	StemMACS CardioDiff Basal Medium XF, human	95
	StemMACS CardioDiff Cardiac Induction Supplement XF (20×), human	5

Table 1: Preparation of complete media.

1. Thaw supplements at +2 to +8 °C overnight.
   ▲ **Note:** If a white precipitate occurs, pipette up and down to dissolve it.
2. Shake the supplements vials vigorously.
3. Prepare complete media according to table 1.
   ▲ **Note:** (Optional) Antibiotic solutions might be added to prevent contamination, e.g. 100 U/mL penicillin and 100 µg/ mL streptomycin.
4. Complete media can be stored for up to 4 weeks at +2 to +8 °C.

2.3 Detailed differentiation protocol

▲ To achieve an efficient differentiation, it is important to start the differentiation with high quality PSCs. Regularly monitor the pluripotency status of the stem cell cultures by observing their morphology and staining for pluripotency markers. The PSCs culture should show a confluency of 75–85% before starting.

▲ The amount of Cardiac Cultivation Medium provided in the kit is sufficient for maintenance up to day 10. For longer cultivation periods, StemMACS Cardiac Cultivation Medium XF, human is available as a single product (# 130-125-287).

The day before starting
Coat 12-well plates with Matrigel according to the manufacturer's recommendation

Day 0
Harvesting and plating of human pluripotent cells
When using a new stem cell clone it is strongly recommended to perform a titration experiment in order to determine the best starting PSC number for differentiation. Suggested cell numbers are 125,000 cells/cm², 250,000 cells/cm², 300,000 cells/cm², and 400,000 cells/cm².

▲ Warm Matrigel-coated plates at room temperature.

▲ Prepare complete media as indicated in table 1.

▲ Volumes given below are for PSCs originally cultivated in a 6-well plate and transferred to a 12-well plate for induction. If using other culture ware adjust the volumes accordingly.

1. Aspirate cell culture medium and wash each well of the 6-well plate with 2 mL of D-PBS without Ca^{2+} and Mg^{2+}.
2. Add 1 mL/well 0.05% Trypsin/EDTA. Gently rock the plate to ensure even distribution of the solution.
3. Incubate for 5 minutes in the dark at +37 °C.
4. Stop enzymatic reaction by adding 1 mL/well of Soybean Trypsin Inhibitor (0.5 mg/mL).
5. Using a 5 mL serological pipette, dissociate to a single-cell suspension by carefully pipetting up and down.
6. Determine the cell number and viability. Viability should be >95%.
7. Transfer the desired cell number into a new tube. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
8. Resuspend the cells in a sufficient amount of Mesoderm Induction Medium. Per well, 2 mL of Mesoderm Induction Medium are needed.
9. Transfer cells into a Matrigel-coated 12-well plate with 2 mL/well. Place the plate into the incubator (+37 °C, 5% CO_2).

Day 1
Replacing medium with Cardiac Cultivation Medium

1. One day (24 hours) after seeding, aspirate Mesoderm Induction Medium from the culture plate.
2. Wash each well with 2 mL D-PBS to remove dead cells.
3. Replace with 2 mL/well of Cardiac Cultivation Medium.

Day 2
Replacing medium with Cardiac Induction Medium

1. One day (24 hours) after previous feeding, aspirate Cardiac Cultivation Medium from the culture plate.
2. Wash each well with 2 mL D-PBS to remove dead cells.
3. Replace with 2 mL/well of Cardiac Induction Medium.

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Day 3
Replacing medium with Cardiac Cultivation Medium

1. One day (24 hours) after previous feeding, aspirate Cardiac Induction Medium from the culture plate.
2. Wash each well with 2 mL D-PBS to remove dead cells.
3. Replace with 2 mL/well of Cardiac Cultivation Medium.

Day 4–8
Exchanging medium with Cardiac Cultivation Medium daily

1. Replace old Cardiac Cultivation Medium with 2 mL/well fresh medium every 24 hours.
2. (Optional) Wash each well with 2 mL D-PBS to remove dead cells.
   ▲ **Note:** Depending on the PSC line, first contractions can be observed already on day 6 of differentiation.

▲ Cells can be maintained in culture for ≥30 days if fed with StemMACS Cardiac Cultivation Medium XF, human (# 130-125-287) every two days.

▲ If cardiomyocytes shall be longer cultured and especially when the cells start to show signs of detachment, it is recommended to transfer them into new Matrigel-coated plates.

Day 10
Harvesting of cells

1. After 10 days, cells can be harvested using 0.5% Trypsin/EDTA. For optimal detachment, it is recommended to use the Multi Tissue Dissociation Kit 3 (# 130-110-204).

3. References

1. van Mil, A. *et al.* (2018) Modelling inherited cardiac disease using human induced pluripotent stem cell-derived cardiomyocytes: progress, pitfalls, and potential. Cardiovasc. Res. 114(14): 1828–1842.
2. Bernstein, D. (2017) Induced pluripotent stem cell-derived cardiomyocytes: a platform for testing for drug cardiotoxicity. Prog. Pediatr. Cardiol. 46: 2–6.
3. Hartman, M. E. *et al.* (2016) Human pluripotent stem cells: Prospects and challenges as a source of cardiomyocytes for *in vitro* modeling and cell-based cardiac repair. Adv. Drug Deliv. Rev. 96: 3–17.

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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