PSC Cardiomyocyte Differentiation Kit

USER GUIDE

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gibco

Print Options

Package contents

Catalog Number	Amount	
A29212-01	1 kit	Kit Contents

Storage conditions

- 2°C to 8°C, Protect From Light

Required materials

- Essential 8™ Medium (Cat. No. A15170)
- Geltrex™ LDEV-Free hESC-Qualified Reduced Growth Factor Basement Membrane Matrix (Cat. No. A1413302)
- DPBS, no calcium, no magnesium (Cat. No. 14190)
- UltraPure™ 0.5 M EDTA, pH 8.0 (Cat. No. 15575)
- Appropriate tissue culture plates and supplies

Additional required materials

Timing

Recovery and Expansion: 4 days

Differentiation: 7–10 days

Selection guide

Stem Cell Differentiation

Go online to view related products.

Product description

The PSC Cardiomyocyte Differentiation Kit is a complete ready-to-use xeno-free system for the efficient differentiation of human pluripotent stem cells (PSCs) into contracting cardiomyocytes within 10 days of initiating differentiation.

Important guidelines

Click here for important procedural guidelines.

Online resources

Visit our product page for additional information and protocols.

For support, visit www.thermofisher.com/support.

Protocol outline

Day 0: Recover PSCs in Essential 8™ Medium on Geltrex™ matrix-coated plate.

Day 1, 2, and 3: Refeed cells every day with Essential 8™ Medium.

Day 4: Refeed cells with Cardiomyocyte Differentiation Medium A.

Day 6: Refeed cells with Cardiomyocyte Differentiation Medium B.

Day 8+: Refeed cells with Cardiomyocyte Maintenance Medium every other day.

Culture conditions

Culture type: Adherent

Recommended substrate: Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix (Cat. No. A14133). For xeno-free applications, use recombinant vitronectin (Cat. No. A14700).

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Limited product warranty, disclaimer, and licensing information

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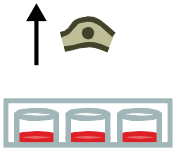
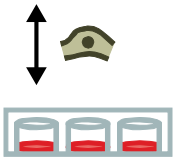
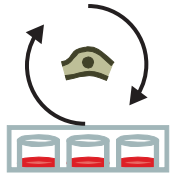
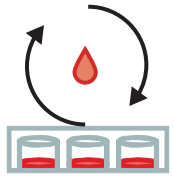
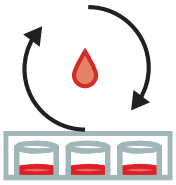
Prepare PSCs for differentiation (Days 0–3)

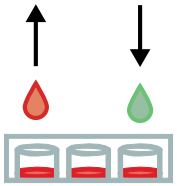
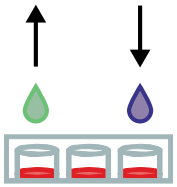
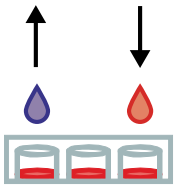
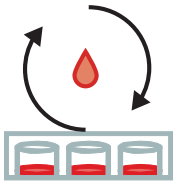
Follow the procedure below to prepare PSCs for differentiation into cardiomyocytes under feeder-free culture conditions. For the differentitation procedure, see page 3.

The volumes given in the protocol are for a 12-well culture plate; for volumes recommended for other vessel sizes, click here.

	Step		Action	Procedure details
Day 0	1		**Harvest PSCs**	**On day 0** (day of splitting), the PSC culture should exhibit 70–85% confluence. Before starting, prepare Geltrex™ matrix-coated 12-well plates as recommended in product manual and equilibrate to room temperature. a. Aspirate the spent medium and rinse each well of the PSC culture plate with 1 mL of DPBS, no calcium no magnesium. b. Aspirate the DPBS, then add add 0.5 mL of pre-warmed TrypLE™ reagent to each well and incubate at 37°C until the cells round up and become detached (approximately 5–7 minutes). **Note:** Observe the cells under a microscope to ensure complete cell detachment from the well surface. Gently tap the plate to dislodge the cells, if necessary. Do not overdigest the cells.
	2		**Generate a single-cell suspension**	a. Add 1 mL of Essential 8™ Medium to each well and pipet the cells up and down 3–5 times to generate single cell suspension. Transfer the cell suspension to a sterile conical tube. b. Centrifuge the cell suspension at 200 × *g* for 4 minutes at room temperature, discard the supernatant, and resuspend the pellet in an appropriate volume of room temperature Essential 8™ Medium containing 1X RevitaCell™ Supplement (approximately 1 mL of medium per well of a 12-well plate).
	3		**Passage PSCs in Essential 8™ Medium**	a. Determine the viable cell density and percent viability using a Countess™ II Automated Cell Counter or similar device/method. At this stage, cell viability is typically >95%. b. Add the appropriate amount of cell suspension into each well of the Geltrex™-coated 12-well plate to achieve 30–70% confluence within four days (2×10^4–1.6×10^5 cells/well). **Note:** For new or uncharacterized cell lines, we recommend a range finding study to determine the optimal confluence at the onset of differentiation (Day 3 or 4). See "Important guidelines" on page 1. c. Incubate the cells overnight at 37°C in a humidified atmosphere of 5% CO_2.
Day 1	4		**Expand PSCs in Essential 8™ Medium**	**On day 1** (about 24 hours after PSC splitting), PSCs should be at 5–15% confluence. a. Aspirate the spent medium and add 1 mL of pre-warmed complete Essential 8™ Medium into each well of the 12-well plate. **Note:** Addition of RevitaCell™ Supplement is not required from this point onward. b. Incubate the cells overnight at 37°C in a humidified atmosphere of 5% CO_2.
Days 2–3	5		**Refeed PSCs with Essential 8™ Medium**	**On days 2 and 3**, refeed the cells with Essential 8™ Medium. a. Aspirate the spent medium and add 1 mL of pre-warmed complete Essential 8™ Medium into each well of the 12-well plate. b. Incubate the cells overnight at 37°C in a humidified atmosphere of 5% CO_2.

Differentiate PSCs into cardiomyocytes (Days 4–12)

Follow the procedure below to differentiate PSCs into cardiomyocytes under feeder-free culture conditions. Differentiated cardiomyocytes can continue to be maintained in Cardiomyocyte Maintenance Medium for >15 days in culture.

	Step		Action	Procedure details
Day 4	6		**Replace medium with Cardiomyocyte Differentiation Medium A**	**On day 4**, the PSC culture should exhibit 30–70% confluence, with a target range of 35–60%. a. Aspirate the spent medium and replace with 1 mL/well of pre-warmed Cardiomyocyte Differentiation Medium A. b. Return cells to the 37°C incubator with a humidified atmosphere of 5% CO_2.
Days 6	7		**Replace medium with Cardiomyocyte Differentiation Medium B**	**On day 6**, the cells will start to become opaque. Some shedding of dead cells is normal. a. Aspirate the spent medium and replace with 1 mL/well of pre-warmed Cardiomyocyte Differentiation Medium B. b. Return cells to the 37°C incubator with a humidified atmosphere of 5% CO_2.
Day 8	8		**Replace medium with Cardiomyocyte Maintenance Medium**	**On day 8**, the cells will continue to become more opaque. Some shedding of dead cells is normal. a. Aspirate the spent medium and replace with 1 mL/well of pre-warmed Cardiomyocyte Maintenance Medium. b. Return cells to the 37°C incubator with a humidified atmosphere of 5% CO_2.
Days 10–12	9		**Refeed every other day with Cardiomyocyte Maintenance Medium**	**On days 10 and 12**, refeed the differentiating cells with Cardiomyocyte Maintenance Medium. Contracting cardiomyocytes can appear as early as day 10. a. Aspirate the spent medium and replace with 1 mL/well of pre-warmed Cardiomyocyte Maintenance Medium. b. Return cells to the 37°C incubator with a humidified atmosphere of 5% CO_2.
Day 14	10		**Characterize cardiomyocytes**	**On day 14**, spontaneously contracting syncytium of troponin T cardiac type 2 (TNNT2/cTnT) positive cardiomyocytes will be present and ready for use in various research applications. We recommend the Human Cardiomyocyte Immunocytochemistry Kit (Cat. No. A25973) for optimal image-based analysis of two key markers of the human cardiac lineage: NKX2-5 for early cardiac mesoderm and TNNT2/cTNT for cardiomyocytes. ⓘ Human Cardiomyocytes stained with the Human Cardiomyocyte Immunocytochemistry Kit.

The following protocols describe the cryopreservation generated cardiomyocytes, recovery of cryopreserved cardiomyocytes, and passaging of hiPSC-derived cardiomyocytes until the cells are ready for use in an assay.

Click here for materials required for cryopreservation, recovery, and passaging of cardiomyocytes, and downstream assays for their characterization.

Cryopreserve cardiomyocytes

The following protocol for the cryopreservation of cardiomyocytes have not been fully optimized for cardiomyocytes, but is provided as a starting point. Select cardiomyocytes that are exhibiting robust contraction over large areas of culture surface before cryopreservation. See Required materials, Additional required materials. For additional details on cryopreserving human PSCs, visit the product page for PSC Cryopreservation Kit (Cat. No. A26446-01).

1. Rinse each well with 1 mL of DPBS (no calcium, no magnesium).
2. Aspirate the DPBS and add 1 mL of pre-warmed TrypLE™ reagent to each well and incubate at 37°C for 5–10 minutes.
3. Resuspend the cells using a 1-mL pipette and transfer the cell suspension into a tube containing 1 mL of Cardiomyocyte Maintenance Medium.
4. Filter the cells using a 100-µM cell strainer, then perform a cell count.
5. Centrifuge the cell suspension at 200 × *g* for 4 minutes and aspirate the medium, being careful not to disturb the cell pellet.
6. Gently resuspend the cell pellet in chilled PSC Cryopreservation Medium (2°C to 8°C) to a concentration of 1×10^7 cells/mL.
7. Dispense aliquots of the cell suspension (mix frequently to maintain a homogeneous cell suspension) into cryovials.
8. Freeze the cells in an automated or manual controlled rate freezing apparatus (1°C decrease per minute).
9. Transfer the frozen cells to liquid nitrogen (vapor phase).

Recover cryopreserved cardiomyocytes

1. Prepare Geltrex™ substrate-coated 12-well plates.
2. Quick-thaw cryopreserved cardiomyocytes in a 37°C water bath until only a small ice crystal remains.
3. Gently resuspend the cells and transfer the cell suspensions into a 15-mL conical tube.
4. Dilute the cell suspension with 10 mL of Cardiomyocyte Maintenance Medium, adding it dropwise to avoid osmotic shock while gently rocking the tube back and forth.
5. Centrifuge the cell suspension at 200 × *g* for 4 minutes.
6. Aspirate the medium, being careful not to disturb the cell pellet.
7. Gently resuspend the cells in Cardiomyocyte Maintenance Medium supplemented with 1X RevitaCell™ Supplement (Cat. No. A2644501).
8. Aspirate Geltrex™ substrate from the newly coated 12-well plate and seed the plate at 0.5×10^7–1×10^7 cells/well.
9. Incubate the cells for 18–24 hours at 37°C with humidified atmosphere of 5% CO_2.
10. Next day, replace the medium with fresh Cardiomyocyte Maintenance Medium (without RevitaCell™ Supplement). Refeed every other day. Cardiomyocytes generally exhibit contracting behavior by day 3 after recovery.

Dissociate and replate hiPSC-derived cardiomyocytes

Note: Ten to fourteen days after the start of differentiation is the ideal time to dissociate cardiomyocytes. Cultures could be dissociated at later times, but the junctions between the cardiomyocytes become stronger over time, making it more challenging to maintain high viability after dissociation.

1. Before starting the dissociation, prepare a multiwell plate of choice to plate the dissociated cardiomyocytes by coating the plate with Geltrex™ matrix using the directions in the manual for pluritpotent stem cells. After adding the Geltrex™ matrix to the plates, allow the Geltrex™ matrix to coat at 37°C for 2 hours in a humidified incubator with 5% CO_2.
2. Starting from a differentiation culture, work with cultures that have spontaneous contraction occurring in at least 50% of the wells around day 10 to day 14 from the start of differentiation.
3. Before dissociation, perform a wash of your differentiated cultures with DPBS (no calcium, no magnesium) by aspirating the PSC Cardiomyocyte Maintenance Medium, filling the well with DPBS (no calcium, no magnesium), then aspirating the DPBS.
4. Add an appropriate volume of TrypLE™ Express Enzyme, for example use 500 µL for one well of a 12-well plate and incubate at 37°C for 10–15 minutes in a humidified incubator with 5% CO_2.
5. After incubation, gently resuspend the cultures using a 1-mL pipette until a single cell solution is obtained. If needed, run the dissociated solution on a cell strainer (~35 µm mesh size) to remove any undissociated clumps.
6. Add 4 volumes of a protein rich medium such as DMEM + 10% FBS or StemFlex™ Medium to neutralize the TrypLE™ Express Enzyme and to avoid cell reaggregation while in suspension. Mix well by pipetting up and down several times.
7. Spin down the cells for 5 minutes at 300 × *g*.
8. Aspirate the supernatant and resuspend the cells in PSC Cardiomyocyte Maintenance Medium containing 1X RevitaCell™ Supplement.
9. Count the cells including Trypan Blue staining using a Countess™ II Automated Cell Counter or equivalent instrument to determine cell count and viability.
10. After cell count, aspirate the Geltrex™ matrix from the pre-coated plates, and plate the dissociated cells at a desired density in PSC Cardiomyocyte Maintenance Medium containing 1X RevitaCell™ Supplement. We recommend a seeding density of between 2×10^5 to 1×10^6 viable cells/cm^2, depending on the downstream application, and we recommend optimizing the seeding density for each assay. For image-based assays, use lower seeding densities; for functional assays, where synchrony of contracting cardiomyocytes is key, seed the cells more densely.
11. One day after plating, exchange the medium with PSC Cardiomyocyte Maintenance Medium only, then continue with regular medium changes thereafter until the cells are ready for use in an assay.

Downstream assays

Examples of downstream assays are high-content assays for cell structure or size (Human Cardiomyocyte Immunocytochemistry Kit, Cat. No. A25973) and cell health (CellEvent™ Caspase 3/7 Green Detection Reagent, Cat. No. C10723; PrestoBlue™ Cell Viability Reagent, Cat. No. A13262; Mitotracker™ dyes and others), or functional assays that visualize the contraction via monitoring calcium (Fluo-4 Direct™ Calcium Assay Kit, Cat. No. F10471) or voltage (FluoVolt™ Membrane Potential Kit, Cat. No. F10488).