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by Thermo Fisher Scientific

PSC Cardiomyocyte Differentiation Kit

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. Differentiated cardiomyocytes can be maintained in Cardiomyocyte Maintenance Medium for >30 days.

Product*	Catalog no.	Amount	Storage
PSC Cardiomyocyte Differentiation Kit contains:	**A29212-01**	**1 Kit**	2°C to 8°C, Protect from light.
Cardiomyocyte Differentiation Medium A	A29209-01	100 mL	
Cardiomyocyte Differentiation Medium B	A29210-01	100 mL	
Cardiomyocyte Maintenance Medium	A29208-01	500 mL	

* The PSC Cardiomyocyte Differentiation Kit is sold as a complete kit; its components are not available separately except for Cardiomyocyte Maintenance Medium, which can be purchased separately for maintaining long-term cultures. For additional protocols and applications refer to **thermofisher.com/stem-cell-differentiation.html**.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Caution: Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV and HBsAg. Handle in accordance with established bio-safety practices.

Culture conditions

Culture type: Adherent

Recommended substrate: For general applications, we recommend Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix (Cat. no. A1413302). For xeno-free applications, we recommend recombinant vitronectin (Cat. no. A14700). Please refer to the substrate product manuals for coating protocols.

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.

Guidelines for differentiation

- Use high quality human PSCs (with minimal or no differentiated colonies) that are karyotypically normal, express pluripotency markers, and are undergoing routine passaging. Do not use a PSC line past 100 passages.
- We recommend culturing PSCs under feeder-free conditions using Essential 8™ Medium (Cat. no. A1517001) on Geltrex™ matrix, which is an ideal substrate surface for cardiomyocyte differentiation. For details on culturing PSCs in Essential 8™ Medium, refer to **thermofisher.com/culturing-puripotent-stem-cells-essential-8-medium**.
- The differentiation efficiency of PSC into cardiomyocytes varies between different PSC lines. A critical variable for the generation of robust cardiomyocyte culture is the relative confluence at the onset of differentiation. We strongly recommend performing a confluence range finding study when using a PSC line for the first time. See Table 2 for guidelines.
- Rapid media aspiration and addition can be detrimental to culture differentiation efficiency; we recommend slow addition and removal of media, especially during the induction phase.
- Addition of 1X RevitaCell™ Supplement (Cat. no. A2644501) is essential for effective survival and recovery of singularized PSCs following seeding under these conditions. Alternatively, ROCK inhibitors (10 µM Y27632 or 0.5 µM Thiazovivin) may also be used.

Differentiate PSCs into cardiomyocytes

1. **PSC culture day 1:** To seed cells for differentiation, start with healthy PSC culture approximately 70%–85% confluent. Prepare Geltrex™ matrix-coated 12-well plates as recommended in product manual and equilibrate to room temperature prior to use.
   Note: For reagent volumes for different culture vessels, see Table 1.
2. Prepare sufficient volume of Essential 8™ Medium with 1X RevitaCell™ Supplement for use in steps 6–8, and warm to room temperature before use.
3. Aspirate the spent medium from healthy PSC culture and rinse wells once with DPBS (without Ca^{2+} and Mg^{2+}).
4. Aspirate the DPBS and add pre-warmed TrypLE™ reagent to each well and incubate at 37°C until cells round up and readily detach by swirling or gentle tapping (approximately 3–5 minutes). Triturate cells 3 to 5 times while rinsing surface of well to help remove and resuspend cells. It is important not to over-digest cells. Microscopic observation to assess detachment is recommended.
5. Transfer cell suspension to a sterile conical tube containing Essential 8™ Medium, volume based values provided in Table 1, and mix by gentle pipetting or inversion.
6. Centrifuge the cell suspension at 200 × *g* for 4 minutes at room temperature, carefully discard the supernatant, gently flick tube 3–5 times to loosen pellet, and resuspend pellet in appropriate volume of Essential 8™ Medium containing 1X RevitaCell™ Supplement.
   Note: 1–2 times the volume of TrypLE™ reagent used for dissociation is a good estimate for Essential 8™ Medium resuspension volume.
7. Determine the viable cell density and percent viability using a Countess™ Automated Cell Counter or similar device/method.
   Note: Cell viability is typically >95%.
8. Aspirate Geltrex™ solution from plate, add Essential 8™ Medium with RevitaCell™ Supplement to wells, and plate cells on tissue culture dish according to the following guidelines for plating cells:
   A. *New or Uncharacterized PSC lines*– we strongly recommend a range finding study to determine optimal confluence levels at onset of differentiation (Day 3 or 4). Refer to guildelines in Table 2 for cell seeding densities and expected confluence ranges using a 12 well plate.
   B. *Characterized PSC lines*– use previously optimized seeding densities
9. **PSC culture day 2:** About 24 hours after plating PSCs, cells should be at 5–15% confluence. Aspirate the spent medium and add pre-warmed complete Essential 8™ Medium into each well. Return the plate to the incubator.
   Note: Addition of RevitaCell™ Supplement or other ROCK inhibitors is not required from this point onward.

10. **PSC culture day 3:** View cells under microscope and estimate confluence (see Figure 1). If the confluence is below target, refeed the cells with pre-warmed Essential 8™ Medium and return the plate to the incubator. Begin differentiation the following day, proceeding to step 11.
    If desired confluence is achieved, proceed to step 11.
11. **Differentiation day 1**: The PSC cultures should exhibit the approximate confluence range specified in Table 2 for new lines, or optimal confluence based on prior range finding. Aspirate the spent medium and slowly add pre-warmed **Cardiomyocyte Differentiation Medium A** into each well. Return the plate to the incubator.
    Note: Throughout differentiation period, change media every 2 days, as indicated.
12. **Differentiation day 3**: The cells will start to become opaque. Some shedding of dead cells is normal. Aspirate the spent medium from each well and slowly replace with pre-warmed **Cardiomyocyte Differentiation Medium B**. Return the plate to the incubator.
13. **Differentiation day 5**: The cells will continue to become more opaque. Shedding of dead cells is normal. Some PSC lines may shed more dead cells than others. Aspirate the spent medium from each well and slowly replace with pre-warmed **Cardiomyocyte Maintenance Medium**. Return the plate to the incubator.
14. **Differentiation days 7, 9 and 11:** Refeed cells with Cardiomyocyte Maintenance Medium. Return the plate to the incubator. Contracting cardiomyocytes can appear as early as day 8.
15. **Differentiation day 12**: Typically a spontaneously contracting syncytium of troponin T cardiac type 2 (TNNT2/cTnT) positive cardiomyocytes will be present and ready for use in various research applications.
    Note: Differentiated cells can be further cultured up to day 12-15 for harvesting (dissociation) and cryopreservation; beyond this time cells are difficult to effectively harvest and recover. Alternatively, the cardiomyocytes can be maintained for a month or more for long-term studies, such as electrophysiological assays or molecular characterization. Additional Cardiomyocyte Maintenance Medium may be required beyond what is provided in this differentiation kit.

Table 1 Reagent volumes per well or dish

Culture vessel (Surface area/well)	DPBS	TrypLE™ Select Enzyme	Essential 8™ Medium
6-well (10 cm^2/well)	2 mL	1 mL	2 mL
12-well (4 cm^2/well)	1 mL	0.5 mL	1 mL
24-well (2 cm^2/well)	0.5 mL	0.25 mL	0.5 mL
35-mm (10 cm^2/dish)	2 mL	1 mL	2 mL
60-mm (20 cm^2/dish)	4 mL	2 mL	4 mL
100-mm (60 cm^2/dish)	12 mL	6 mL	12 mL

Table 2 Rangefinding: Seeding density vs. confluence on day 4

Culture vessel (Surface area/well)	Viable cells/well	% confluence on "day 4"
12-well (4 cm^2/well)	2×10^4	30%
	3×10^4	50%
	4×10^4	70%
	5×10^4	90%

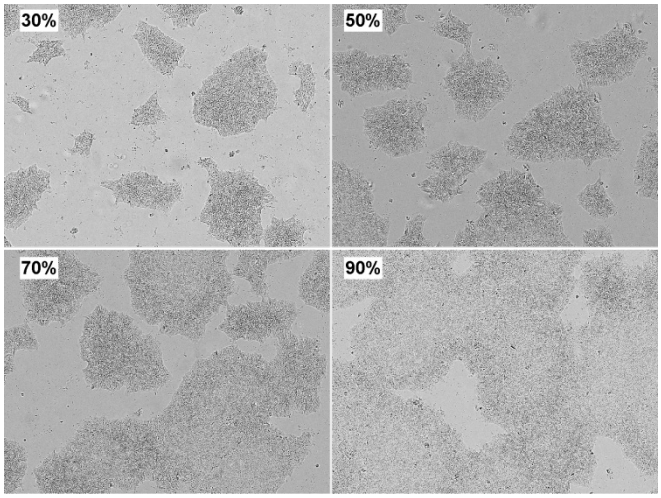

Figure 1 Images of H9 cultures 4 days following seeding at densities described in Table 2. Confluence measurements were performed using automated image analysis and values indicated in upper left corner of each panel. For this set of H9 cultures, optimal confluency was about 60–80%.

Related products

Product	Cat. no.
Essential 8™ Medium	A1517001
DPBS, no calcium, no magnesium	14190
TrypLE™ Select Enzyme (1X), no phenol red	12563
Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix	A14133
Vitronectin, truncated human recombinant (VTN-N)	A14700
RevitaCell™ Supplement	A26445
Human Cardiomyocyte Immunocytochemistry Kit	A25973

Explanation of symbols and warnings

The symbols present on the product label are explained below:

Caution, consult accompanying documents	Temperature Limitation	Keep away from light	Use By:	Consult instructions for use
LOT	REF		STERILE A	Read SDS
Batch Code	Catalog number	Manufacturer	Sterilized using aseptic processing techniques	Read Safety Data Sheet

Limited product warranty

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