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Single cell dissociation of PSCs for cardiomyocyte differentiation

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Introduction

This protocol supplements the PSC Cardiomyocyte Differentiation Kit Prototype user guide (Pub. no. MAN0010627). Refer to the user guide for the description of the PSC Cardiomyocyte Differentiation Kit Prototype (Cat. no. A25042SA) and for detailed instructions on differentiating pluripotent stem cells (PSCs) into contracting cardiomyocytes under feeder-free culture conditions.

Materials Needed

- Pluripotent stem cells (PSCs) to differentiate
- Essential 8® Medium (Cat. no. A1517001)
- RevitaCell™ Supplement (100X) (Cat. No. A26445-01)
- Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium (Cat. no. 14190)
- Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix (Cat. no. A1413302) for general applications or Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Cat. no. A14700) for xenofree applications
- TrypLE™ Select Cell Dissociation Reagent (Cat. no. 12563)
- Sterile cell culture hood (i.e., biosafety cabinet)
- Inverted microscope
- Incubator set at 37°C, 5% CO_2
- Water bath set at 37°C
- Sterile serological pipettes (5-mL, 10-mL)
- Centrifuge
- 15-mL centrifuge tubes
- Appropriate tissue culture plates and supplies

Guidelines for maximizing the differentiation potential of PSCs into cardiomyocytes

- It is critical to use high quality human PSCs (with minimal or no differentiated colonies) that are karyotypically normal, confirmed to exhibit pluripotency markers, and are routinely passaged every three days for at least three passages before starting cardiomyocyte differentiation. Additionally, we recommend that PSC line not be used past 100 passages.
- Depending on the cell reprogramming technology, incomplete conversion of adult cell type into induced pluripotent state may lead to the generation of refractory cell lines that are unable to differentiate into mesodermal lineage cell types. We recommend inclusion of a positive control cell line like the H7 or H9 hESC line to assess the ability of your iPSC line to differentiate into beating cardiomyocytes.
- Singularizing PSCs for differentiation to cardiomyocytes allows better seeding and confluence estimates, resulting in more consistent results well-to-well and overall better differentiation of difficult to differentiate lines. We recommend the protocol below for singularizing PSCs for differentiation into cardiomyocytes.

Single cell dissociation procedure

1. Prepare 1:100 Geltrex® matrix solution (for general applications) in Essential 8® medium or 1:50 vitronectin solution (for xeno-free applications) in Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium. Coat your culture vessel with your matrix of choice (see Table 1, below, for the recommended volume) and incubate for one hour at 37°C.
2. Prepare Essential 8® Medium with 1X RevitaCell™ Supplement (Cat. no. A26445-01) and warm to room temperature before use.

   Note: Addition of the RevitaCell™ Supplement is critical for effective survival and recovery of PSCs after seeding.
3. Aspirate the spent medium from your PSC culture and rinse wells with Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium (see Table 1, below).
4. Aspirate the DPBS and add pre-warmed TrypLE™ reagent to each well (see Table 1, below) and incubate at 37°C until the cells 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.
5. Add Essential 8® Medium to each well and pipet the cells up and down about 3 times to generate single cell suspension. Transfer the cell suspension to a 15-mL conical tube.
6. Centrifuge the cell suspension at 200 × *g* for 5 minutes, discard the supernatant, flick the tube 3–5 times to loosen the pellet, and resuspend the cell pellet with the appropriate amount of Essential 8® Medium.
7. Determine viable cell density and percent viability using a Countess® Automated Cell Counter (you may also use a similar automated or manual method).
8. Aspirate the coating solution (Geltrex® or vitronectin) from the coated culture vessel and add an appropriate amount of Essential 8® Medium supplemented with 1X RevitaCell™ Supplement.
9. Seed the PSCs into the coated culture vessel at the appropriate seeding density. See Table 1, below, for the recommended seeding density for your specific culture vessel.
10. Incubate the cells overnight in a 37°C incubator with 5% CO_2.

    Note: It is typical to observe small star-shaped colonies on the culture surface. This morphological characteristic will disappear within a day or two after the initial seeding.
11. Following incubation, aspirate the Essential 8® Medium supplemented with 1X RevitaCell™ Supplement and refeed the cultures with unsupplemented Essential 8® Medium (without the addition of RevitaCell™ Supplement) every day until the desired confluence is reached within 2 to 4 days (30–70% confluence).
12. Follow the cardiomyocyte differentiation protocol.

Table 1 Volume of diluted vitronectin solution needed to coat various culture vessels.

Culture vessel	Geltrex® solution in Essential 8® Medium (1:100)	Vitronectin solution in DPBS (1:50)	DPBS	TrypLE™ reagent	Essential 8® Medium	Total number of cells
6-well (10 cm^2/well)	1 mL	1 mL	2 mL	1 mL	2 mL	5×10^4–4×10^5 cells/well
12-well (4 cm^2/well)	0.5 mL	0.5 mL	1 mL	0.5 mL	1 mL	2×10^4–1.6×10^5 cells/well
24-well (2 cm^2/well)	0.25 mL	0.25 mL	0.5 mL	0.25 mL	0.5 mL	1×10^4–8×10^4 cells/well
35-mm (10 cm^2)	1 mL	1 mL	2 mL	1 mL	2 mL	5×10^4–4×10^5 cells/dish
60-mm (20 cm^2)	2 mL	2 mL	4 mL	2 mL	4 mL	1×10^5–8×10^5 cells/dish
100-mm (60 cm^2)	6 mL	6 mL	12 mL	6 mL	12 mL	3×10^5–2.4×10^6 cells/dish

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