gibco

USER BULLETIN

Enrichment and Replating of PSC-Derived Cardiomyocytes

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Introduction

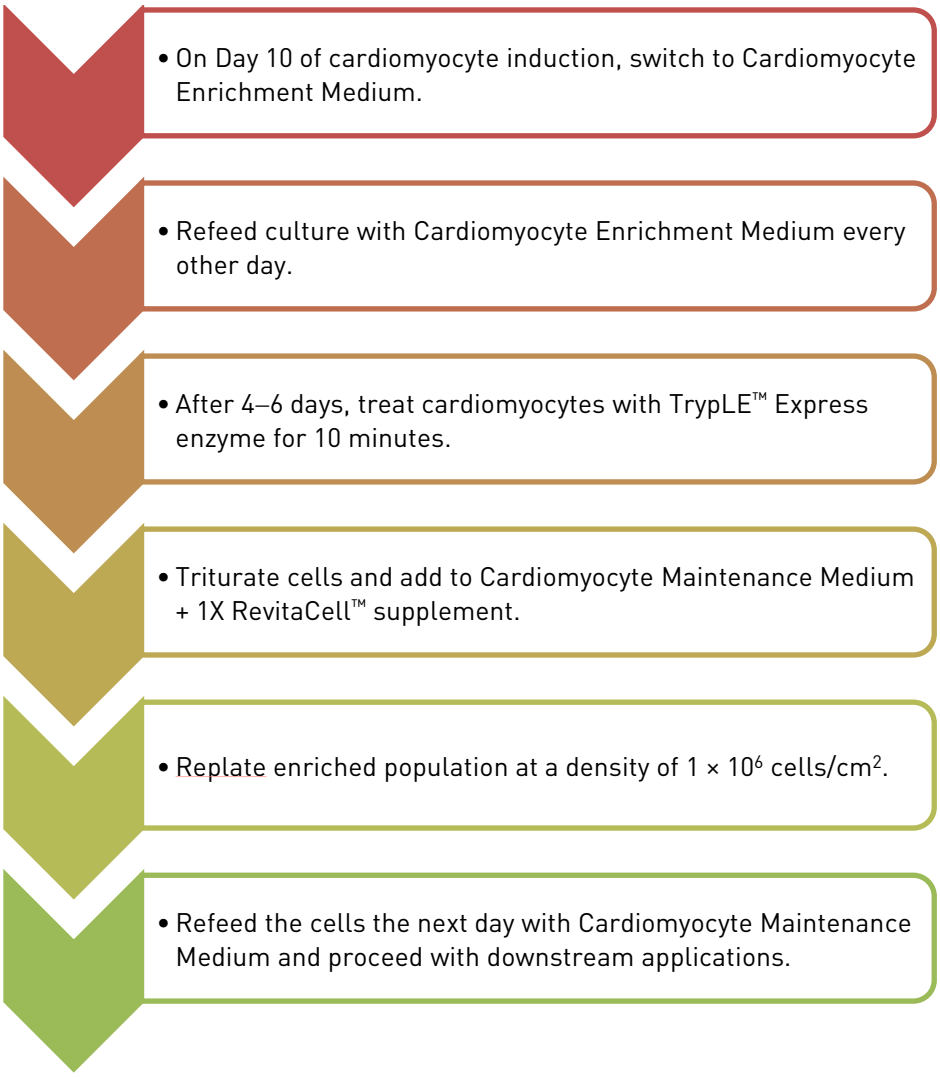
This protocol supplements the **PSC Cardiomyocyte Differentiation Kit user guide** (Pub. no. MAN0014509). Refer to the user guide for the description of PSC Cardiomyocyte Differentiation Kit (Cat. no. A29212-01) and for detailed instructions on inducing human pluripotent stem cells (PSC) into contracting cardiomyocytes.

The following protocol is based on metabolic selection and replating of cardiomyocytes described in Tohyama *et al.* (Cell Stem Cell, 2013) and Burridge *et al.* (Nat Methods, 2014), which can yield an enriched population of cardiomyocytes, especially with PSC lines that exhibit moderate cardiomyocyte differentiation efficiency. There is a substantial loss of non-cardiomyocyte cells and some TNNT2+ cardiomyocytes when using this protocol.

Materials Needed

- RPMI 1640 Medium, without glucose (Cat. no. 11879-020)
- Bovine Albumin Fraction V (7.5% solution) (Cat. no. 15260-037)
- HEPES (1M) (Cat. no. 15630-106)
- Sodium Lactate (Syrup, 60% w/w), Fisher Chemical (Fisher Scientific, Cat. no. S326-500)
- L-Ascorbic acid sodium salt, 99%, ACROS Organics™ (Fisher Scientific, Cat. no. AC35268-1000)
- Dulbecco's Phosphate Buffered Saline (DPBS), without calcium and magnesium (Cat. no. 14190-250)
- UltraPure™ DNase/RNase-Free Distilled Water (Cat. no. 10977-023)
- TrypLE™ Express Enzyme (1X), no phenol red (Cat. no. 12604-054)
- PSC Cardiomyocyte Maintenance Medium (Cat. no. A29208-01)
- Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix (Cat. no. A14133-02)
- RevitaCell™ Supplement (100X) (Cat. no. A26445-01)
- 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

Figure 1. Workflow for the enrichment and replating of PSC-derived cardiomyocytes.



Prepare media and reagents

250X ascorbic acid solution (10 mL)

1. To prepare 10 mL of ascorbic acid solution at a final concentration of 5 mM, aseptically mix the following components:

Ascorbic acid	1 g
UltraPure™ DNase/RNase-Free Distilled Water	6 mL

2. Dissolve ascorbic acid until it goes into solution. Heating or vortexing may be required.
3. Once dissolved into solution, add remaining balance of water to QS to 10 mL.
4. Aliquot and store at –20°C, protected from light, for up to 6 months. Once thawed, use immediately; do not store at 4°C.

1 M lactate solution (10 mL)

1. To prepare 10 mL of lactate solution at a final concentration of 1 M, aseptically mix the following components:

Sodium Lactate 60%	1.43 mL
1 M HEPES	8.57 mL

2. Once mixed, store at 4°C, protected from light, for up to 6 months.

Cardiomyocyte enrichment medium (CEM) (100 mL of complete medium)

1. To prepare 100 mL of complete cardiomyocyte enrichment medium (CEM), aseptically mix the following components:

RPMI 1640 Medium without glucose	96.17 mL
Bovine Albumin Fraction V (7.5% solution)	3.3 mL
Sodium Lactate syrup 60%	0.4 mL
250X ascorbic acid solution	0.13 mL

2. Sterilize through 0.22-µm filter and store at 4°C for up to 2 weeks.

Enrichment and replating procedure

The volumes given in the following adaptation procedure are for 12-well plates. For culture vessels with different sizes, adjust the volumes appropriately. See Figure 1, page 2, for an overview of the protocol

1. After PSC have been induced for ten days with the PSC Cardiomyocyte Differentiation kit, aspirate the PSC Cardiomyocyte Maintenance Medium (CMM) and slowly add 1 mL per well of pre-warmed Cardiomyocyte Enrichment Medium (CEM).
2. Place the culture plates in the 37°C, 5% CO_2 incubator and incubate. Refeed the plates every other day with 1 mL of CEM per well.
3. From day four to six, prepare a 1:100 Geltrex™ matrix solution in CMM. Coat your culture plates and incubate for one hour at 37°C.
4. Aspirate the spent CEM from the culture plate and wash the wells once with DPBS without Calcium and Magnesium.
5. Aspirate the DPBS without Calcium and Magnesium, and add 1 mL per well of pre-warmed TrypLE™ Express enzyme.
6. Place the culture plates in the 37°C, 5% CO_2 incubator and incubate for 7–10 minutes.
7. Using a 1-mL pipette, triturate the cardiomyocyte cultures 10 to 15 times to generate a homogenous single-cell suspension.

   Note: If you continue to observe cell clumps, pass the cell suspension through a 100-µm cell filter.
8. Transfer the single-cell suspension to a sterile15-mL tube with two volumes of CMM with 1X RevitaCell™ supplement.
9. Take an aliquot of the cell suspension, perform a cell count, and calculate the total number of cells.
10. Centrifuge the cells at 200 × *g* for 5 minutes.
11. Aspirate supernatant, gently flick tube 2–3 times to loosen the cell pellet, and resuspend the cells in an appropriate volume of CMM + 1X RevitaCell™ supplement to generate a 4×10^6 cells/mL suspension.
12. Aspirate the Geltrex™ matrix solution from prepared plates and seed enriched cardiomyocytes into wells at density of 1×10^6 cells/cm^2 (1 mL per well of a 12-well plate).

13. Place the culture plates in the 37°C, 5% CO_2 incubator and incubate overnight.

14. Aspirate the spent CMM + 1X RevitaCell™ supplement and replace it with fresh CMM before proceeding with downstream applications. Anticipated yields from this protocol range between 5–35% (see Table 1, below).

Table 1. Anticipated yield of cardiomyocytes after enrichment and replating.

Quality of culture at Day 10	Enrich	Re-plate	Approximate number of TNNT2+ cardiomyocytes (1 well of a 12-well plate)	Approximate yield
<30% TNNT2+ cardiomyocytes	Yes	Yes	150,000	5%
30%–70% TNNT2+ cardiomyocytes	Yes	Yes	180,000–400,000	5–15%
>70% TNNT2+ cardiomyocytes	Limited improvement	Yes	400,000–1,000,000	20–35%

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