StemRD
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PSdif-Cardio® Cardiomyocyte Differentiation Kit

PSdif-Cardio® *is designed for cardiomyocyte differentiation of human pluoripotent stem cells (ESC or iPSC) grown as a monolayer culture. The kit contains all necessary serum-free media formulated with polypeptide differentiation factors and modulators of the key cardiogenic pathways for a 3-step derivation of beating cardiomyocytes. It is optimized to use with the serum-free and feeder-free growth medium* ***PSGro®*** *(StemRD cat# PGro). In independent beta testing of multiple human ESC and iPSC lines, the kit consistently generated cardiomyocytes at efficiencies higher than published monolayer protocols.*

Package Size, Content and Storage

PSdif-Cardio® has two package sizes:

- **Starter** kit: catalog # PC-STR (or PC-000 for samples): 3 wells (6-well plate), ~1x10^7 starting cells
- **Regular** kit: catalog # PC-REG: 15 wells (6-well plate), ~5x10^7 starting cells

Components (5)	*Intended Outcome*	***Starter*** *Kit Size*	***Regular*** *Kit Size*
PSGro® Plus	ESC or iPSC preparation	30 mL	130 mL
PSdif-Cardio® A	mesendoderm derivation	10 mL	50 mL
PSdif-Cardio® B	cardiac mesoderm derivation	10 mL	50 mL
PSdif-Cardio® C	cardiac progenitor derivation	10 mL	50 mL
CardioGro®	maturation & maintenance	50 mL	250 mL

Storage: 2 to 8°C. Keep from light. Do NOT freeze. **Shelf Life:** 1 month if stored as directed.

Other reagents required:

1. **Matrigel™**: for cell plating
2. **Accutase** or equivalent: for cell splitting
3. Phosphate Buffered Saline (**PBS**): for cell washing
4. ROCK inhibitor **Thiazovivin** (StemRD # Thia) or Y-27632 (# Y27632): for optimal cell plating
5. **PSGro®** (StemRD # PGro) or equivalent (e.g., mTeSR®): for hESC/iPSC maintenance (*optional*)

Cell Preparation in PSGro® Plus

Coating plates with Matrigel™: *Refer to manufacturer's instruction.*

Recovery of frozen cells in PSGro®: *Refer to PSGro® User Manual on StemRD website for details.*
https://www.stemrd.com/index.php?/Media/PSGro-Medium/flypage.tpl.html

Adaptation of growing cells to PSGro®

Most human ESC or iPSC lines that have been cultured as feeder-dependent or feeder-independent culture can be adapted to PSGro®. Refer to PSGro® User Manual on StemRD website for details.

Cell plating in PSGro® Plus

1. Start from a routine culture of hESC or hiPSC in **PSGro®** or a similar serum-free medium (e.g., mTeSR®). Identify and remove differentiated cells by scraping and aspiration.
2. Aspirate the medium and rinse twice with PBS.
3. Add 0.5 mL of Accutase per well (6-well plate). Incubate at 37°C for 3 – 5 min and verify that colonies have become single cells or small clusters (2 – 10 cells/cluster) under microscope.
4. Add 2 mL/well **PSGro® Plus** and pipet up & down 2 – 3 times gently.
5. Transfer the detached cells to a conical tube. Centrifuge at 200 x *g* for 5 minutes at room temp.
6. Aspirate the supernatant. Resuspend pellet in 3 mL **PSGro® Plus** gently. *Note: adding* ***Thiazovivin*** *(2.5 uM) or* ***Y-27632*** *(10 uM) to* ***PSGro® Plus*** *at this step markedly increases plating efficiency.*
7. Plate the cells in a Matrigel™-coated well. For most cell lines, a 1:4 to 1:10 splitting from a routine, sub-confluent, culture may be appropriate while the ideal split may vary between lines.
8. Culture at 37°C, 5% CO_2 / 95% humidity. Refresh with **PSGro® Plus** (without ROCKi) daily.

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Differentiation with PSdif-Cardio®

Cell density at the onset of differentiation: *cell density is critical to achieve optimal cardiomyocyte differentiation. The ideal cell density at the onset of induction is **95 - 100%** confluency. Less confluent culture may suffer from excessive cell loss upon induction whereas over-confluent cultures may acquire undesired differentiation before induction. Usually, if cells are plated in **PSGro® Plus** as recommended, they should reach 95 - 100% confluency in 3 – 4 days. A good prediction of this optimal density is cells reaching around 80% confluency on the day before.*

A. Warm **PSdif-Cardio® A** to room temp. Aspirate PSGro® Plus, rinse once with PBS. Add 3 mL of **PSdif-Cardio® A**, incubate the cells at 37°C, 5% CO_2/95% humidity for **1** day (20 to 28 hr).

Expected result: As a result of differentiation, certain degree of cell dislodging may occur. Excessive cell loss is usually due to lower than ideal starting cell density. If more than half of the cells are lost, differentiation is likely to fail. Increase starting cell density in the next round.

B. Warm **PSdif-Cardio® B** to room temp. Aspirate **PSdif-Cardio® A**, rinse once with PBS. Add 3 mL **PSdif-Cardio® B**, incubate the cells for **2** days. Do not change the medium.

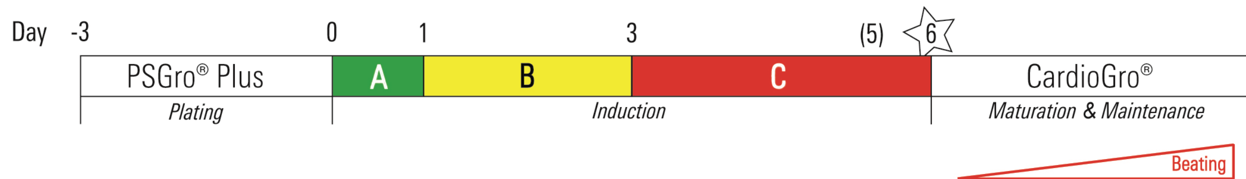
Expected result: As a result of differentiation, cell shape changes. Cell dislodging may continue.

C. Warm **PSdif-Cardio® C** to room temp. Aspirate **PSdif-Cardio® B**, rinse once with PBS. Add 3 mL **PSdif-Cardio® C**, incubate the cells for **3** days. Do not change the medium.

*Expected result: Cell dislodging and shape change continue, meanwhile, continuing proliferation may increase confluency. **NOTE**: This step can be shortened to **2** days (if the culture appears exhausted) without appreciable loss of efficiency.*

D. Warm **CardioGro®** to room temp. Aspirate **PSdif-Cardio® C**, rinse once with PBS. Add 3 mL **CardioGro®**, and incubate the cells for 2 – 3 days. Refresh the medium every 2 – 3 days.

*Expected result: Clusters of beating cells typically appear after 1 – 4 day in **CardioGro®** (day 7 – 10 from induction) and expand over the next few days. Cell clustering and detachment from the plate may occur. Survival and maturation of cardiomyocytes are expected over several weeks.*



Trouble-shooting

1. Excessive amount of cell dislodging or death at **Step A**: This usually occurs when cell density is too low at the onset of induction. Do not start the induction before cells reach 95% to 100% confluence. Less cell dislodging is expected for **over**-confluent culture, albeit efficiency may or may not decrease.

2. Low efficiency: A large number of factors influence cardiogenic efficiency of a particular pluoripotent stem cell line. The main determinants include:

- The well-known diversity in cardiogenic capacities between pluoripoent cell lines, especially hiPSC lines: Different cell lines may require optimization in starting cell density, length of the 3 steps, and addition of other agents not included in this kit (see Burridge, Cell Stem Cell, 10: 16, 2012). Since ESC lines are generally more amenable to differentiation, a strongly-cardiogenic ESC line should be included as a control.
- The quality of the starting cells: it is crucial to maintain cells at their full pluripotent state before induction.

3. No beating cell: If no beating cell appears after **3 weeks** despite of optimization, the procedure has very likely failed. As cardiogenic differentiation is governed by a large number of factors, many of which are out of StemRD's or operators' control, success cannot be guaranteed for all cell lines under every laboratory setting.

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