Ncardia
Stem cell experts

Ncyte Cardiomyocytes

User Guide
Version 1 - March 2020

Contents

1. General Information

This protocol covers thawing, seeding and maintenance when using Ncyte Cardiomyocytes. Please read the entire protocol before starting the study.

2. Safety Information

- Ncyte Cardiomyocytes are intended for *in vitro* research use only.
- The product is not intended for diagnostics, therapeutic or clinical use and is not approved for human *in vivo* applications.
- Cardiomyocytes can be inactivated by autoclaving at 121 °C for 20 minutes.
- Cardiomyocytes should be cultured in a sterile environment.
- Gloves and lab coats should be worn when handling all reagents.

3. Materials

3.1 Cells and medium provided

Material	Order number	Container	Storage
hiPSC-derived Cardiomyocytes	Nc-C-BRCM	1x Cryo vial	Liquid nitrogen
Cardiomyocyte Culture Medium	Nc-M-CMCM-250	1 x Bottle	+4 °C

3.2 Storage conditions

Cryopreserved cells: Upon receipt of cryopreserved cardiomyocytes, transfer the vials directly to the vapor phase of liquid nitrogen for further storage. Do not expose the vials to room temperature and do not store cells at -80°C, as recrystallization will harm the cells.

Medium: Store frozen Cardiomyocyte Culture Medium at -20°C upon receipt. Thaw medium overnight at 4°C. Avoid excessive exposure to light. Once thawed, medium can be kept at 4°C for up to 4 weeks

3.3 Required consumables

Materials	
Sterile disposable 5 ml pipettes	
Sterile 50 ml conical tubes	
Sterile filter tips for pipettes	
T75 flask	
Reagents	**Vendor / Catalog number**
DPBS (+$Mg^{2+}$$Ca^{2+}$)	Gibco, Cat. No. 14040
DPBS	Gibco, Cat. No. 14190
Fibronectin (1 mg/ml)	Sigma-Aldrich, Cat. No. F1141
Cardiomyocyte Culture Medium	Nc-M-CMCM-250, 250 mL bottle
Cardiomyocytes	Ncardia, 1x cryovial
TrypLE Express	Thermo Fisher, 100 ml, no phenol red; Cat. No. 12604013
Y-27632	Axon-1683

3.4 Required equipment

Equipment
Flow cabinet
Incubator at 37°C, 5% CO_2 and humidified air
Water bath at 37°C
Centrifuge
Pipettes (P10, P20, P1000)
Multichannel pipette (30-300 µl)

4. Methods

4.1 Coating of T75 flasks

1. Dilute fibronectin 1:100 in DPBS (+Mg^{2+} Ca^{2+}) to get a 10 µg/ml fibronectin coating solution. Mix the solution gently.
2. Add 15 ml fibronectin coating solution per T75 flask. **Note**: One T75 is required per vial.
3. Incubate the flask(s) for at least 1.5 hours at 37°C, 5% CO_2 and humidified air.

4.2 Thawing and seeding

4. Pre-warm 30 ml of Cardiomyocyte Culture Medium to room temperature for thawing, rinsing and plating. Add 10 µM Y27632 to the medium for the first 24 hours after thawing and plating.

5. Add 12 ml of room temperature Cardiomyocte Culture Medium into a new 50 ml conical tube.

6. Transfer the vial of Cardiomyocytes from liquid nitrogen storage to a 37°C water bath and thaw for 3 minutes (until only a small ice clump remains).

7. Wipe the vial with 80% ethanol and transfer immediately to the laminar flow hood.

8. Transfer the 2 mL cell suspension from the vial into the conical tube.

9. Rinse the Cardiomyocyte vial with 1 ml of room temperature culture medium. Transfer the solution into the 50 ml conical tube . Final volume should be 15 ml.

10. Centrifuge the cell suspension at 250xg for 3 minutes.

11. Discard the supernatant. Resuspend the cells in 15 ml fresh, room temperature culture medium.

12. Aspirate the coating solution gently from the T75 flask(s) and add the cell suspension.

13. Incubate the flask(s) at 37°C, 5% CO_2 and humidified air for 24 hours.

4.3 Maintenance of T75 flask

14. Pre-warm Cardiomyocte Culture Medium (without Y27632) to 37˚C in a water bath.
Important: This step requires use of Cardiomyocyte Culture Medium without Y27632.

15. Aspirate the old medium and refresh with 15 ml culture medium (37˚C) per T75 flask.

Refreshment schedule: Day 1 and Day 3 post-thaw. Day 3: refresh at least 2 hours before dissociation.

4.4 Coating assay plate

16. Dilute fibronectin 1:100 in DPBS (+Mg^{2+} Ca^{2+}) to get a 10 µg/ml fibronectin coating solution. Mix the solution gently.

17. Transfer the solution into a multichannel reservoir.

18. Add 50 µl fibronectin coating solution per well to a 96-well plate using a multichannel pipette.

19. Incubate the plate(s) for at least 3 hours at 37°C, 5% CO_2 and humidified air.

4.5 Dissociation and seeding in assay plate

20. Aspirate the medium from the T75 flask.

21. Wash the cells with 15 ml DPBS without Ca^{2+}/Mg^{2+} per T75.

22. Add 3 ml 1x TrypLE Express per T75 flask.

23. Incubate for 10 minutes in the incubator at 37°C and check the detachment of the cells after the first 5 minutes. It is recommended to tap the flask gently to support dissociation. Do not exceed 10 minutes.

24. Neutralize the dissociation process with 3 ml Cardiomyocyte Culture Medium (37°C) per T75 flask and transfer into a 50 ml conical tube.

25. Rinse each flask with 3 ml Cardiomyocyte Culture Medium (37°C) and transfer into the same tube. Total volume should be 9 ml.

26. Collect samples for counting live/dead cells using a manual counting method (e.g. Fuchs Rosenthal Counting Chamber).

27. Centrifuge the cell suspension at 250xg for 3 minutes.

28. Discard the supernatant and resuspend the cells in fresh Cardiomyocyte Culture Medium to the desired concentration. See Table 1 for recommended volumes and plating densities.

    Example: 30,000 cells/100 µl for a 96-well plate.

29. Aspirate the excessive coating solution in the plate(s).

30. Seed the cells into the plates(s).

31. Leave the plate at room temperature for 30 min in order to let the cells settle down.

32. Incubate the plate(s) at 37°C, 5% CO_2 and humidified air for 24 hours.

Culture device	Surface Area (cm^2)	Seeding Volume (ml)	Cell Number ~94,000 / cm^2
6-well Cell Culture Plate	9.5	2.5	9.0×10^5
12-well Cell Culture Plate	3.8	1.0	3.6×10^5
24-well Cell Culture Plate	1.9	0.5	1.8×10^5
96-well Cell Culture Plate	0.32	0.1	3.0×10^4
384-well Cell Culture Plate	0.06	0.025	5.6×10^3
T25 Flask	25	6.5	2.4×10^6

Table 1. Recommended seeding volumes and plating densities. Consult Ncardia Support at support@ncardia.com for advice, if needed.

4.6 Maintenance of assay culture

33. Pre-warm Cardiomyocyte Culture Medium to 37˚ in a water bath.

34. Aspirate the old medium from the plate and refresh with new Cardiomyocyte Culture Medium (37°C).

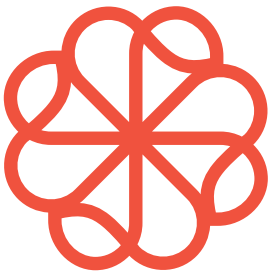
Recommended refreshment schedule: D1 post-seed and subsequently every other day until assay day. On the assay day, refresh at least 1 hour prior to the assay.

To eliminate weekend handling steps: refresh late Friday afternoon (twice the regular volume) and next Monday morning (normal volume).

5. Technical Support

Ncardia is ready to help you with any questions regarding this User Guide or Ncyte Cardiomyocytes.

E-mail: support@ncardia.com
Telephone: +31 (0) 71 332 2230
(Monday - Friday, 09:00 - 17:00 CET)



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