Ncardia

User guide

Ncyte® Cardiac Fibroblasts

Human iPSC-derived cardiac fibroblasts

User guide UG-833

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Contents

Getting Started

Please make sure to read the entire User Guide carefully before you start thawing and culturing Ncyte® Cardiac Fibroblasts.

A Material Safety Data Sheet (MSDS) for Ncyte® products is available on www.ncardia.com or upon request to support@ncardia.com.

The information in this guide is subject to change without notice.

Technical Support

If you need further assistance contact our team at support@ncardia.com.

1. General information

This protocol covers thawing, seeding, and culturing of Ncyte® Cardiac Fibroblasts. Please read the entire protocol before using this product.

Ncyte® Cardiac Fibroblasts are produced through a well-defined *in vitro* differentiation process from human induced pluripotent stem cells (iPSC). The iPSC line is generated by introducing specific transcription factors, described by Yamanaka, using a non-viral system.

Ncyte® Cardiac Fibroblasts express typical cell markers, such as VIM and TCF21

2. Safety Information

- Ncyte® Cardiac Fibroblasts 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.
- Ncyte® Cardiac Fibroblasts can be inactivated by autoclaving at 121 °C for 20 minutes.
- Ncyte® Cardiac Fibroblasts should be cultured in a sterile environment.
- Gloves and lab coats should be worn when handling all reagents.

It is highly recommended to wear ISO 374 certified gloves and lab coats when handling all reagents, as some reagents contain chemicals that may be harmful. Please consult the product's Certificate of Analysis (CoA) and Material Safety Data Sheet (MSDS) for further information and safety instructions.

3. Materials

3.1. Cells and medium provided by Ncardia

Material	Order number	Container	Content	Storage
Ncyte® Cardiac Fibroblasts	M0833	Cryovial	≥ 0.4 M cells	Vapor phase of liquid nitrogen
Ncyte® Cardiomyocyte Culture Medium (Optional)	M0822	1 bottle	250 mL	-20°C

Table 1: Overview of Ncyte® Cardiac Fibroblasts content.

3.2. Storage conditions

Cryopreserved cells: Upon receipt, directly transfer the vials to the vapor phase of liquid nitrogen for further storage. Do not expose the vials to room temperature. 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

Consumables	Vendor	Cat. No.
Tissue culture ware	Various	-
Sterile 50 mL polypropylene tubes	Various	-
DPBS +/+	Various	-
DPBS (+Mg2+Ca2+)	Gibco	14040
DPBS (no calcium, no magnesium)	Gibco	14190
Fibronectin (1 mg/mL)	Sigma	F1141
FGF2 (10 µg/mL)	Miltenyi	130-093-840
Ciprofloxacin (2 mg/mL)	Sigma	17850
TrypLE Express 100 ml, no phenol	Thermo Fisher	12604013

Table 2: Overview of required consumables

3.4. Required equipment

Item	Vendor
Flow cabinet Bio Safety Cabinet Class II	Various
Incubator at 37°C, 5% CO_2 and humidified air	Various
Water bath at 37°C	Various
Centrifuge	Various
Pipettes (P10, P20, P1000)	Various
Multichannel pipette (30-300 µl)	Various

Table 3: Overview of required equipment

4. Preparation

4.1. Medium

Ciprofloxacin solution – 2 mg / mL

Cardiomyocyte Culture Medium does not contain antibiotics. We recommend adding Ciprofloxacin (2mg/mL) to the 250 mL bottle of Cardiomyocyte Culture Medium at a 1000-fold dilution (e.g. add 250 µL to 250 mL).

FGF2 - 10 µg/mL

Prepare a 10 µg/mL stock solution of FGF2 according to the manufacturer's instructions. Add to the culture medium to achieve a final concentration of 5 ng/mL (1:2000 dilution).

4.2. Surfaces

Ncyte® Cardiac Fibroblasts can be cultured on numerous surfaces.

Plastic: Ncyte® Cardiac Fibroblasts adhere best on cell culture-treated plastic surfaces. Plasticware from Nunc™ (Nunclon Delta Surface) or Greiner (Greiner Bio-One®) are recommended.

4.3. Coating

Choice of coating depends on the intended assay. As a standard coating for cultivation of Ncyte® Cardiac Fibroblasts, it is recommended to use Fibronectin.

Coating plates with Fibronectin

Note: Do not allow the Fibronectin coating to dry out.

1. Dilute sterile Fibronectin to 5 µg/mL in DPBS with Ca2+ and Mg2+.
2. Add a proper volume of Fibronectin solution to cover the bottom of the culture vessel (see Table 4).
3. Incubate the culture vessel in a cell culture incubator at 37°C for 2-3 hours.
4. Aspirate Fibronectin solution immediately before seeding.

Plate format	Volume coating (mL)	Volume culture medium (mL)
T75 flask	15	30
T25 flask	5	10
6-well plate	2	4
12-well plate	1	2
24-well plate	0.5	1
48-well plate	0.25	0.5
96-well plate	0.1	0.2

Table 4: Suggested coating and culture medium volumes per culturing format

5. Cell Culture

5.1. Thawing

Note:

- For transportation of frozen vials from a liquid nitrogen storage tank to the cell culture room, a dewar filled with liquid nitrogen should be used. Do not use dry ice for transportation because it may affect cell viability.
- Repeated pipetting, vigorous shaking or vortexing may damage thawed cells.
- Use Ncyte® Cardiomyocyte Culture Medium supplemented with Ciprofloxacin and FGF2 to thaw and maintain the cells.

1. Coat a 6-well plate as described in section 4.2
2. Pre-warm the required volume of Cardiomyocyte Culture Medium to 37°C.
3. Add 5 mL of Cardiomyocyte Culture Medium into a 15 mL conical tube.
4. Quickly transfer the vial of Ncyte® Cardiac Fibroblasts from liquid nitrogen storage to a 37°C water bath and thaw until only a small ice clump remains.
5. Wipe the vial with 70% ethanol and transfer immediately to the laminar flow hood.
6. Transfer the cell suspension from the vial into the conical tube from step 5.1.3.
7. Rinse the of Ncyte® Cardiac Fibroblasts vial with 1 mL of Cardiomyocyte Culture Medium. Transfer the solution into the 15 mL conical tube.
8. Centrifuge the cell suspension at 300xg for 3 minutes at room temperature..
9. Discard the supernatant and resuspend the cell pellet in 2 mL of fresh Cardiomyocyte Culture Medium.
10. Determine the number of live/dead cells using a manual or automated counting method (e.g. Fuchs-Rosenthal Counting Chamber or automated counting such as Nucleocounter® NC-200).

5.2. Seeding for pre-culture

1. Plate 200,000 cells in 2 mL per well for a 6-well plate (~21,000 cells/cm2)
2. Aspirate the coating solution gently from the coated culture ware and add the recommended volume of cell suspension.
3. Incubate the plate(s) at 37°C, 5% CO2 and humidified air overnight.

5.3. Maintenance

1. Always refresh the cells on Day 1 post-thaw, as follows:
2. Pre-warm the required volume of Ncyte® Cardiomyocyte Culture Medium supplemented with Ciprofloxacin and FGF2 to room temperature.
3. Aspirate the old medium and refresh with the recommended volume of culture medium (Table 4).

Note:

- During this pre-culture period, the cells continue proliferating and the confluency of the culture will increase. Cells are typically harvested on Day 3 post-thaw for seeding assay plates.

5.4. Dissociation and seeding in assay plate

1. Coat the desired assay plate as described in section 4.2
2. We recommend to dissociate the cells on Day 3 post thaw, as follows:
3. Pre-warm the required volume of Cardiomyocyte Culture Medium to 37˚C in a water bath.
4. Aspirate medium from the culture ware and wash once with DPBS (no calcium, no magnesium). Example: Use 2 mL per well for a 6-well plate.
5. Add 1 mL of TrypLE Select and incubate for 5 minutes at 37°C, 5% CO2.
6. Neutralize by adding 1 mL Cardiomyocyte Culture Medium.
7. Detach the cells from the surface of the wells by pipetting up and down 3x with a P1000 and collect the cells in a 50 mL conical tube
8. Wash culture well with 1 mL with Cardiomyocyte Culture Medium in order to collect residual cells and transfer them into the same 50 mL conical tube.
9. Centrifuge the cell suspension at 300xg for 3 minutes at room temperature.
10. Discard the supernatant and resuspend the cell pellet in 1 mL of fresh Cardiomyocyte Culture Medium.
11. Determine the number of live/dead cells using a manual or automated counting method (e.g. Fuchs-Rosenthal Counting Chamber or Nucleocounter® NC-200).
12. Adjust the cell suspension to the desired concentration in Cardiomyocyte Culture Medium based on the downstream assay. For example: 5,000-10,000 cells in 0.2 mL per well for a 96-well plate. Optimal density should be adjusted for different applications.
13. Aspirate the coating solution gently from the coated cultureware and add the recommended volume of cell suspension.
14. Incubate the plate(s) at 37°C, 5% CO2 in a humidified atmosphere.

Note:

- Let plates stand horizontally during seeding to avoid an uneven distribution of cells. Keep the tips of the pipette close to the bottom of the plate when seeding to reduce the risk of formation of air bubbles.

5.5. Maintenance of assay culture

We recommend changing the culture medium one day after plating followed by refreshes every other day using pre-warmed Ncyte® Cardiomyocyte Culture Medium supplemented with Ciprofloxacin and FGF2, until the day of the assay. Remove 100% of spent medium and add the corresponding amount of fresh medium (Table 4).

6. Appendix

6.1. Morphology in culture

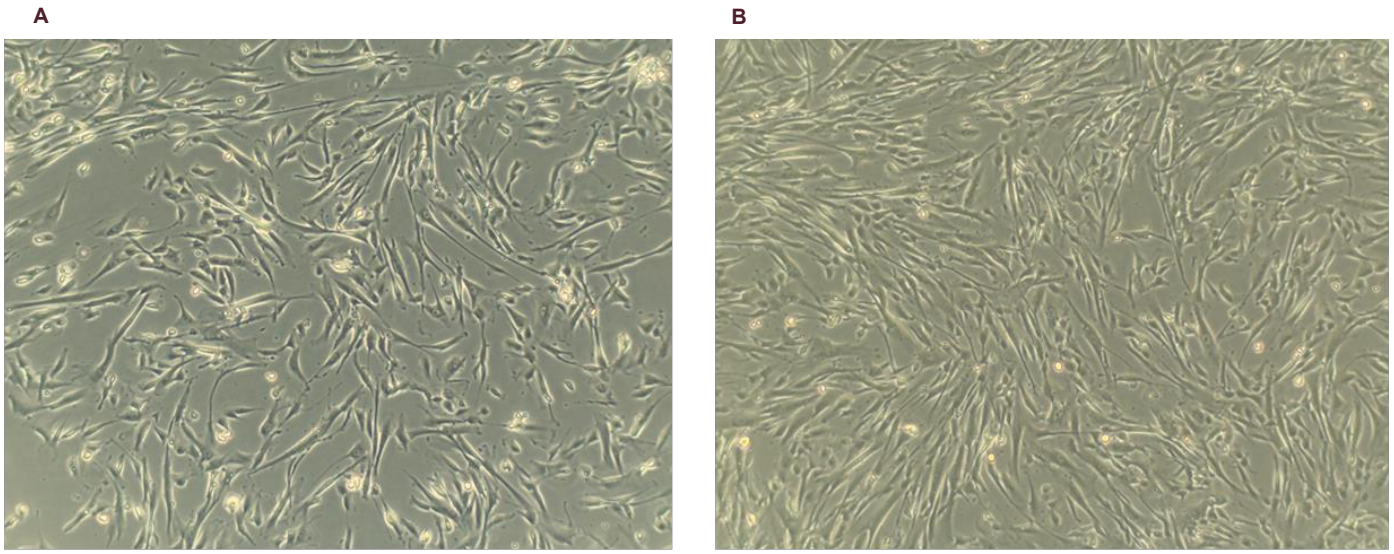

Figure 1: Ncyte® Cardiac Fibroblasts morphology at 1 day (A) and 3 days (B) days after seeding, 4Xmagnification.

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