Ncardia

User guide

Ncyte® NHP-C vCardiomyocytes

Non-Human Primate Cynomolgus iPSC-derived ventricular cardiomyocytes

User guide UG-644

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Ncardia

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Getting Started

Please make sure to read the entire User Guide carefully before you start thawing and culturing Ncyte® NHP-C vCardiomyocytes.

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.

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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® NHP-C vCardiomyocytes. Please read the entire protocol before using this product.

Ncyte® NHP-C vCardiomyocytes. are produced through a well-defined *in vitro* differentiation process from Non-Human Primate Cynomolgus (NHP-C) induced pluripotent stem cells (iPSC). The iPSC line was generated by introducing specific transcription factors, using episomal vectors.

Ncyte® NHP-C vCardiomyocytes express typical cell markers, such as cardiac Troponin T (cTnT) and Alpha-Actinin.

2. Safety Information

- Ncyte® NHP-C vCardiomyocytes. 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® NHP-C vCardiomyocytes are genetically modified cells and therefore genetically modified organisms (GMO). They should be handled according to local directives (Biosafety level 1, US-CDC, or S1, GenTSV, Germany).
- Ncyte® NHP-C vCardiomyocytes can be inactivated by autoclaving at 121°C for 20 minutes.
- Ncyte® NHP-C vCardiomyocytes should be cultured in a sterile environment.

It is highly recommended to wear gloves and lab coat 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

Ncyte® NHP-C vCardiomyocytes are supplied cryopreserved in a vial containing at least 4 million cells.

Material	Cat. No.	Container	Content	Storage
Ncyte® NHP-C vCardiomyocytes	M0644	1 cryovial	≥ 4 million cells	Vapor phase of liquid nitrogen
Ncyte® Cardiomyocyte Culture Medium (Optional)	M0822	1 bottle	250 mL	-20°C

Table 1. Overview of Ncyte® NHP-C vCardiomyocytes content.

3.2. Storage conditions

Cells: Upon receipt of cryopreserved Ncyte® NHP-C vCardiomyocytes, 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

Consumables	Vendor	Cat.No.
Sterile disposable stripettes	Various	-
Sterile 50 mL polypropylene tubes	Various	-
Sterile filter tips for pipette	Various	-
Sterile reservoirs	Various	-
Sterile Eppendorf tubes (1.5 mL)	Various	-
DPBS (+$Mg^{2+}Ca^{2+}$)	Gibco	14040
DPBS (-$Mg^{2+}Ca^{2+}$)	Gibco	14190
Fibronectin (1 mg/mL)	Sigma	F1141
Ciprofloxacin	Sigma	17850
1x TrypLE	Gibco	12563
Y-27632	Axon	1683
Cytoview Maestro MEA 96-well plate	Axion Biosystems	M768-tMEA-96B

Table 2. Overview of required consumables

3.4. Required equipment

Item	Vendor
Flow cabinet	Various
Incubator at 37°C, 5% CO_2 and humidified air	Various
Water bath at 37°C	Various
Centrifuge	Various
Inverted microscope	Various
Cell thawing device	Eppendorf
Pipette controller	Various
Pipettes (P10, P20, P200, P1000)	Various
Multichannel pipette (30-300 µL)	Various
Automated mono channel pipette	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).

4.2. Surfaces

Ncyte® NHP-C vCardiomyocytes adhere best on cell culture-treated plastic surfaces. Plasticware from Nunc™ (Nunclon Delta Surface) or Greiner (Greiner Bio-One®) are recommended.

4.3. Coating

As a standard coating for Ncyte® NHP-C vCardiomyocytes cultivation, we recommend using Fibronectin.

Coating plates with Fibronectin

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

1. Dilute sterile Fibronectin to 10 µg/mL in DPBS with Ca^{2+} and Mg^{2+}.
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 2-3hours.
4. Aspirate Fibronectin solution immediately before seeding.

Plate format	Volume coating (mL)	Volume culture medium (mL)
T25 flask	5	5
96-well plate	0.1	0.1

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

4.4. Coating of MEA plate

1. This protocol must be started ~2 hours before cells are being processed for seeding to allow enough time for processing cells during the total 3 hours incubation time for MEA plates.
2. Dilute fibronectin stock 1 mg/mL 20x in DPBS (with Ca^{2+} and Mg^{2+}) in a microtube to 50 µg/mL solution.

Reagent	Volume (in µL)
Fibronectin solution	25
DPBS+/+	475

Table 5: Suggested volumes of reagents for coating of one MEA plate.

3. Carefully mix by inverting 3 times. Note: Fibronectin is sensitive to shear stress. Do not vortex or spin the solution and avoid harsh pipetting.
4. Gently dispense 5 µL of the diluted fibronectin exactly to the center of the well with an automated mono channel pipette; avoid scratching electrodes with pipet tip.
5. Ensure that the droplet size is as uniform and centered (on the electrodes) as possible.
6. Once all wells are filled with a droplet, add ~4 mL DPBS to the plate grid to keep the plate from drying out.
7. Incubate the plates for 3 hours at (humidified) 37˚C, 5% CO_2.

5. Cell Culture

5.1. Applications of the product

Ncyte® NHP-C vCardiomyocytes are suitable for Multielectrode Array (MEA) assays. The cells can be pre-cultured for two days, dissociated and seeded in MEA plates (Pre-culture), or the cells can be seeded in MEA plates immediately after thaw (Plate Ready). In addition, these cells can be used as Plate Ready cells in Immunocytochemistry (ICC).

5.2. Thawing

Note: One vial of Ncyte® NHP-C vCardiomyocytes is enough to seed one T25, two standard 96-well MEA plates or one 96-well plate for ICC at the recommended cell densities.

1. Pre-warm 20 mL of Cardiomyocyte Culture Medium (supplemented with Ciprofloxacin) to 37 °C for thawing, rinsing and plating. Add 10 µM Y-27632 to the medium for the first 24 hours after thawing and plating.
2. Add 8 mL of room temperature Cardiomyocyte Culture Medium into a new 50 mL conical tube.
3. Transfer the vial of Ncyte® NHP-C vCardiomyocytes from liquid nitrogen storage to a Cell thawing device at 37°C/300rpm and thaw for 4-4.5 minutes (until only a small ice clump remains).
4. Disinfect the vial with 70% ethanol and transfer immediately to the laminar flow hood.
5. Transfer the 1 mL cell suspension from the vial into the conical tube from Step 2.
6. Rinse the of Ncyte® NHP-C vCardiomyocytes vial with 1 mL of Cardiomyocyte Culture Medium. Transfer the solution into the 50 mL conical tube from Step 2.
7. Centrifuge the cell suspension at 250xg for 3 minutes.
8. Aspirate the supernatant. Gently resuspend the cells in 1 mL Cardiomyocyte Culture Medium with a P1000.
9. Add an additional 4.3 mL Cardiomyocyte Culture Medium with a pipette controller.
10. Collect samples for counting live/dead cells using a manual counting method or automated cell counter (e.g. Nucleocounter® NC-200).
11. Count the cell suspension and proceed with cell seeding in a T25 flask (see 5.3), in MEA plates (see 5.6) or in 96-well plates for Immunocytochemistry (See 5.8).

5.3. Seeding of T25 flask

1. Seed 1.8E+05 cells/cm^2 in a volume of 5 mL per T25 flask.
2. Aspirate the coating solution gently from the T25 flask(s) and add the cell suspension.
3. Incubate the flask(s) at 37°C, 5% CO_2 and humidified air until the next morning.

5.4. Maintenance of T25 flask

1. Refresh the cells on Day 1 post-thaw. On Day 2, the cells should be refreshed at least 2 hours before dissociation and replating.
2. Pre-warm Cardiomyocyte Culture Medium (without Y-27632) to 37 ℃ in a water bath for 30 minutes.
   Important: This step requires use of Cardiomyocyte Culture Medium without Y-27632.
3. Aspirate the old medium and refresh with 5 mL Cardiomyocyte Culture Medium (37 ℃) per T25 flask.

5.5. Dissociation of T25 flask

1. Warm Cardiomyocyte Culture Medium to 37ºC in water bath, for 30 minutes.
2. Warm 1x TrypLE to 37ºC in water bath, for 10 minutes.
3. Aspirate the medium from the T25 flask.
4. Wash the cells with 5 mL DPBS without Ca^{2+}/Mg^{2+}per T25.
5. Add 2 mL 1x TrypLE (pre-warmed to 37ºC) per T25 flask.
6. 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.
7. Neutralize the dissociation process by adding 3 mL Cardiomyocyte Culture Medium (37°C) per T25 flask and transfer into a 50 mL conical tube.
8. Rinse each flask with 3 mL Cardiomyocyte Culture Medium (37°C) and transfer into the same tube. Total volume should be 8 mL.
9. Collect samples for counting live/dead cells using a manual counting method (e.g. Fuchs-Rosenthal Counting Chamber) or automated cell counter (e.g. NC-200).
10. Count the cell suspension and proceed with cell seeding in MEA plates (see 5.6).

5.6. Droplet seeding for MEA plate

1. Transfer the required number of cells to a sterile 1.5 mL Eppendorf tube.
2. Centrifuge the cell suspension at 250xg for 3 minutes.
3. Discard supernatant and resuspend the cells in fresh Cardiomyocyte Culture Medium to the desired concentration. See table 6 for recommended volumes and plating densities when plating right after thawing and plating after 2 days pre-culture in a 96-well MEA plate.

Timepoint seeding	Surface area (cm^2)	Seeding volume (µL)	Cell number
After thaw (Plate-Ready)	0.32 (0.08 for recording area)	5 µL droplet per well	10,000 cells per droplet (per well)
After 2 days pre-culture	0.32 (0.08 for recording area)	5 µL droplet per well	5,000 cells per droplet (per well)

Table 6. Recommended cell densities and seeding volumes for a 96-well MEA plate.

4. Remove MEA plate from incubator.
5. Remove coating of 2 columns at a time using a vacuum system and a Pasteur (glass) pipette.
6. Invert the tube with the cells three times to homogenously mix the cells in suspension. Immediately disperse 5 µL cells onto the electrodes with an automated mono channel pipette.
   Note: Avoid touching the coated spot with the pipette tip. Work in a rapid manner.
7. For every 2 columns, repeat removal of the coating solution and the droplet cell seeding. Prior to seeding into each column, gently invert the tube with the cells three times.
8. When all wells are filled, place the plate back to the incubator at 37°C/5%CO_2 and continue incubation for another 2 hours.
9. After 2 hours, remove the plate from the incubator and with a 12-channel pipet, gently add 100 µL of Cardiomyocyte Culture Medium (37°C) along the wall of each well.
10. Transfer plates to humidified incubator at 37°C/5%CO_2 and incubate until the next day (morning).

5.7. Maintenance of MEA plates

1. Pre-warm Cardiomyocyte Culture Medium to 37˚C in a water bath, for 30 minutes.
2. Aspirate the spent medium from the plate and refresh with new Cardiomyocyte Culture Medium (37°C). Add 100 µL per well for the 96-well MEA plate.

We recommend to refresh culture medium on Day 1 after seeding and subsequently every other day until the assay day, which is Day 6 after seeding in MEA plates when using the cells as Plate Ready and Day 4 after seeding in MEA plates for Ncyte® NHP-C vCardiomyocytes that were pre-cultured for 2 days. The cells should exhibit beating within 72 hours after seeding.

5.8. Seeding for immunocytochemistry in 96-well plate

1. Seed 40K-60K cells in a volume of 100 µL per well of a 96-well plate.
2. Aspirate the coating solution gently from the 96-well plate and add the cell suspension.
3. Incubate the 96-well plate at 37°C, 5% CO_2 and humidified air until the next morning.
3. On day 1 after seeding, Aspirate the spent medium from the plate and refresh with new Cardiomyocyte Culture Medium (37°C). Add 100 µL per well of a 96-well plate.
4. On day 2 after seeding, cells can be fixed in 4% Paraformaldehyde and used for Immunocytochemistry assays.

6. Appendix

6.1. Morphology in culture

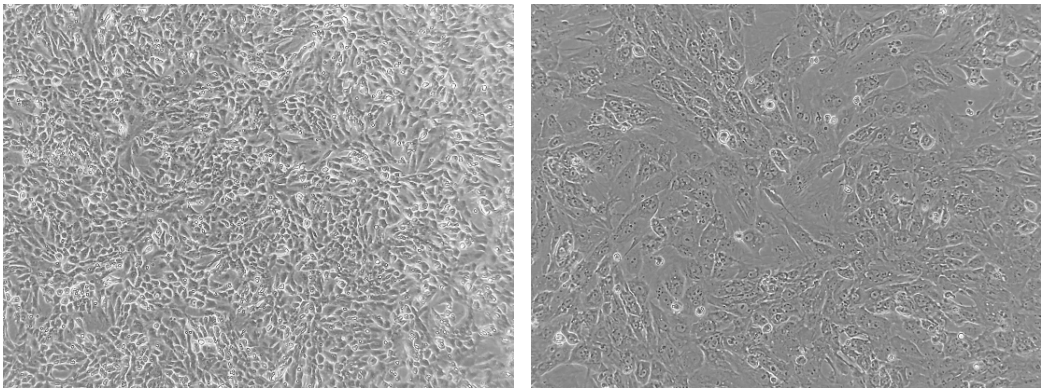

Figure 1: Ncyte® NHP-C vCardiomyocytes morphology 2 days after thawing onto a T25 flask. Images taken at 4X objective (left) and 10X objective (right).

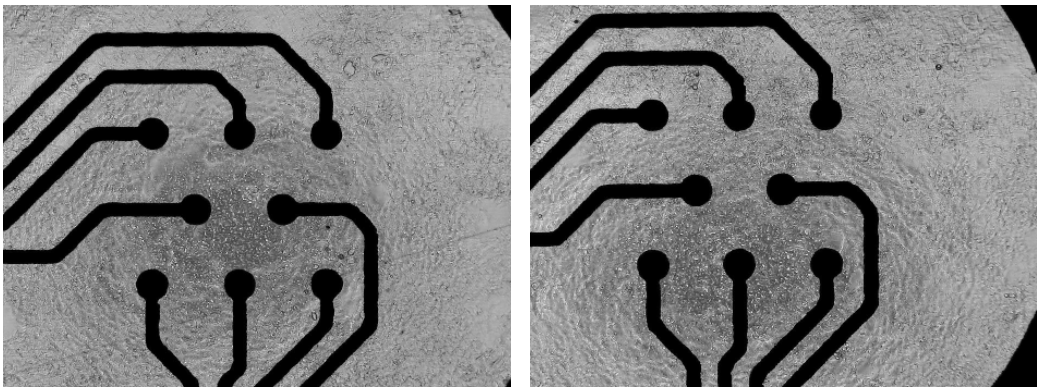

Figure 2: Ncyte® NHP-C vCardiomyocytes morphology 6 days after thawing onto the MEA plate (left) and 4 days after seeding onto the MEA plate after 2 days of pre-culture (right). Images taken at 4X objective.

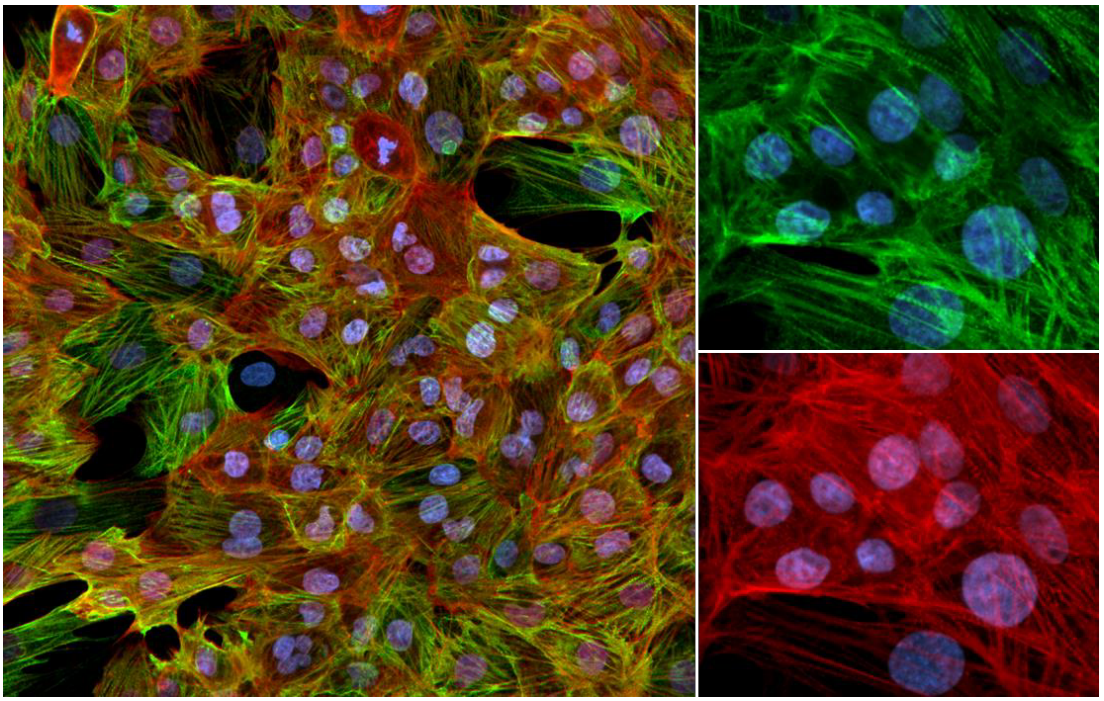

Figure 3. Immunofluorescence staining of Ncyte® NHP-C vCardiomyocytes: Alpha-actinin (Red), cardiac Troponin T (Green) and DAPI (Blue). 40x magnification.

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