Ncardia

User guide

# Ncyte® plate-ready vCardiomyocytes

## Plate-ready human iPSC-derived ventricular cardiomyocytes

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## Getting Started

Please make sure to read the entire User Guide carefully before you start thawing and culturing Ncyte® Plate-ready 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® Plate-ready vCardiomyocytes. Please read the entire protocol before using this product.

Ncyte® Plate-ready vCardiomyocytes 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® Plate-ready vCardiomyocytes express typical cell markers, such as cTnT and MLC2v. These cells are ready to be directly seeded for assays. This user guide describes the protocol for seeding cells for Multielectrode array (MEA) and ICC analysis.

# 2. Safety Information

- Ncyte® Plate-ready 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® Plate-ready vCardiomyocytes can be inactivated by autoclaving at 121°C for 20 minutes.
- Ncyte® Plate-ready vCardiomyocytes should be cultured in a sterile environment.

It is highly recommended to wear 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

Ncyte® Plate-ready vCardiomyocytes are supplied cryopreserved in a vial containing at least 1.5 million cells.

| Material | Cat. No. | Container | Content | Storage |
|---|---|---|---|---|
| Ncyte® Plate-ready vCardiomyocytes | M0848 | 1 cryovial | ≥ 1.5 million cells | Vapor phase of liquid nitrogen |
| Ncyte® Cardiomyocyte Culture Medium (optional) | M0822 | 1 bottle | 250 mL | -20 °C |

**Table 1:** Overview of Ncyte® Plate-ready vCardiomyocytes content.

## 3.2. Storage conditions

**Cryopreserved cells:** Upon receipt of cryopreserved Ncyte® Plate-Ready 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 5 ml pipettes | Various | - |
| Sterile 50 mL polypropylene tubes | Various | - |
| Sterile filter tips for pipette | Various | - |
| DPBS (+Mg2+Ca2+) | Gibco | 14040 |
| DPBS | Gibco | 14190 |
| Fibronectin (1 mg/ml) | Sigma | F1141 |
| Cardiomyocyte Culture Medium | Ncardia | M0822 |
| Ciprofloxacin | Sigma | 17850 |
| Y-27632 | Axon | 1683 |
| Cytoview Maestro MEA 96-well plate | Axion Biosystems | M768-tMEA-96B |
| Cell culture microplate, 384 well | Greiner | 781091 |

**Table 2:** Overview of required consumables

### 3.4. Required equipment

| Item | Vendor |
|---|---|
| Flow cabinet | Various |
| Incubator at 37°C, 5% CO2 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).

## 4.2. 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 3hrs incubation time for MEA plates.
2. Dilute fibronectin stock 1 mg/mL 20x in DPBS (with Ca2+ and Mg2+) 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 monochannel 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% $CO_2$.

## 4.3. Coating of 384 well ICC plate

1. This protocol must be started ~1 hour before cells are being processed for seeding to allow enough time for processing cells during the incubation time.
2. Dilute fibronectin 1:100 in DPBS (+Mg2+ Ca2+) to get a 10 µg/mL fibronectin coating solution.

| Reagent | Volume |
|---|---|
| Fibronectin solution | 200 µL |
| DPBS+/+ | 19.8 mL |

**Table 6**: Suggested volumes of reagents for coating of one 384 well 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. Transfer the solution into a multichannel reservoir.
5. Add 50 µL fibronectin coating solution per well to a 96-well plate using a multichannel pipette.
6. Incubate the plate(s) for at least 3 hours at 37°C, 5% CO2 and humidified air.

# 5. Cell Culture

## 5.1. Thawing and seeding

**Note:** One vial of Ncyte® Plate-ready vCardiomyocytes is enough to seed one standard 96 well MEA plate and one standard 384 well plate at the recommended cell densities.

1. Pre-warm 30 mL of Cardiomyocyte Culture Medium (supplemented with Ciprofloxacin) to room temperature for thawing, rinsing and plating. Add 10 µM Y-27632 to the medium for the first 24 hours after thawing and plating.
2. Add 12 mL of room temperature Cardiomyocyte Culture Medium into a new 50 ml conical tube.
3. Transfer the vial of Ncyte® Plate-Ready vCardiomyocytes from liquid nitrogen storage to a 37°C water bath and thaw for 2-3 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® Plate-Ready vCardiomyocytes vial with 1 mL of room temperature culture medium. Transfer the solution into the 50 mL conical tube from Step 2. Measure the total volume and take a sample for counting live/dead cells using a manual or automatic counting method.
7. Determine the number of live/dead cells using a manual or automated counting method (e.g. Fuchs-Rosenthal Counting Chamber or NC-200 Nucleocounter device) with the sample taken in step 6.
8. Centrifuge the cell suspension at 250xg for 3 minutes.
9. Discard the supernatant and resuspend the cells in fresh Cardiomyocyte Culture Medium to the desired concentration. See Table 7 for recommended volumes and plating densities. Example: $2x10^6$ cells per mL for a 96-well MEA plate. Prepare cell suspension for seeding in a 1.5mL Eppendorf tube for ease of mixing and seeding.

| Culture vessel | Surface area ($cm^2$) | Seeding volume (µL) | Cell number |
|---|---|---|---|
| 96 well MEA plate | 0.32<br>(0.08 for recording area) | 5 µL droplet per well | 10,000 cells per droplet (per well) |
| 384 well ICC plate | 0.06 | 50 µL per well | 9500 cells per 50 µL (per well) |

**Table 7:** Recommended cell densities and seeding volumes for standard assay plates.

**Droplet seeding for MEA plate*:***

1. Remove MEA plate from incubator.
2. Remove coating of 2 columns at a time using a vacuum system and a Pasteur (glass) pipette.
3. Flick the tube with the cells twice at the bottom to homogenously mix the cells in suspension. Immediately disperse 5 µL cells onto the electrodes with an automated monochannel pipette.
   Note: Avoid touching the coated spot with the pipette tip. Work in a rapid manner.
4. For every 2 columns, repeat removal of the coating solution and the droplet cell seeding. Prior to seeding into each column, gently flick the tube with the cells twice.
5. When all wells are filled, place the plate back to the incubator at 37°C/5%CO2 and continue incubation for another 2 hours.
6. After 2 hours, remove the plate from the incubator and with a 12-channel pipet, gently add 100 µL of medium along the wall of each well.
7. Transfer plates to humidified incubator at 37°C/5%CO2 and incubate until the next day (morning).

**Seeding of Ncyte® vCardiomyocytes in 384 well plate**

1. If seeding cells in a 384 well plate, add the calculated volume of Cardiomyocyte Culture Medium using a P200 pipette followed by the volume of cell suspension to the corresponding wells to ensure a cell density of 9500 cells per well.
2. Centrifuge the 384 well plates at 250xg for 3 minutes.
3. Incubate plates at 37°C/5% CO2 overnight.

### 5.2. Maintenance

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 and 50 µL per well for the 384 well plate.

We recommend to refresh culture medium on Day 1 after seeding and subsequently every other day from Monday until Friday, until the assay day. On Friday afternoon, refresh using twice the regular volume, followed by refreshing with the regular volume on Monday morning. On the assay day, refresh at least 1 hour prior to the assay.

# 6. Appendix

### 6.1. Morphology in culture

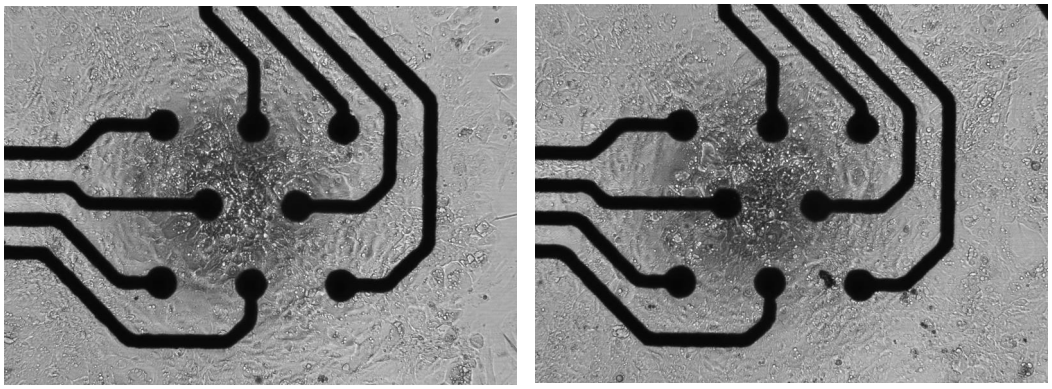
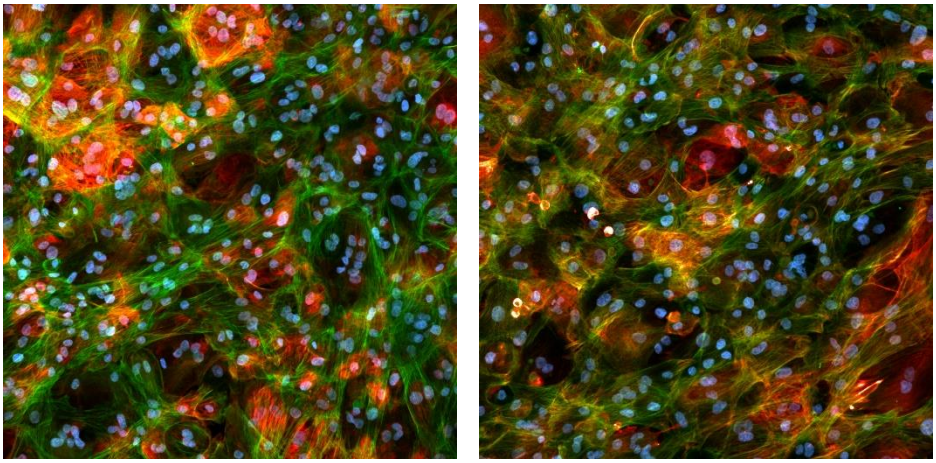


Figure 1: Ncyte® Plate-ready vCardiomyocytes morphology 8 days after thawing onto the MEA plate. Images taken at 4X objective.



CTnT MLC2v CTnT ACTN2

Figure 2: Ncyte® Plate-ready vCardiomyocytes express sarcomere marker Cardiac Troponin T (cTnT), Myosin Light Chain 2v (MLC2v) suggesting a chamber-specific ventricular-like phenotype 8 days after thawing onto the 384 well plate. The cells also express α-Actinin 2, a sarcomeric component in cardiac myocytes. Images taken at 20X objective.

Ncardia

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