Ncardia

User guide

Ncyte® Endothelial Cells

Human iPSC-derived vascular endothelial cells

User guide UG-826

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Getting Started

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

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® Endothelial Cells. Please read the entire protocol before using this product.

Ncyte® Endothelial Cells 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® Endothelial Cells express typical endothelial cell markers, such as CD31 and CD144.

2. Safety Information

- Ncyte® Endothelial Cells 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® Endothelial Cells are genetically modified human 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® Endothelial Cells can be inactivated by autoclaving at 121°C for 20 minutes.
- Ncyte® Endothelial Cells 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® Endothelial Cells are supplied cryopreserved in a vial containing at least 1 million cells.

Material	Cat.No.	Container	Content	Storage
Ncyte® Endothelial Cells	M0826	1 cryovial	≥ 1 million cells	Vapor phase of liquid nitrogen
Ncyte® Cardiomyocyte Culture Medium (Optional)	M0822	1 bottle	250 mL	-20°C

Table 1: Overview of Ncyte® Endothelial Cells 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	-
Fibronectin (1 mg/mL)	Sigma	F1141
VEGF165 (100 µg/mL)	R&D Systems	293-VE
Ciprofloxacin (2mg/mL)	Sigma	17850

Table 2: Overview of required consumables

3.4. Required equipment

Item	Vendor
37°C water bath	Various
Laminar flow hood	Various
Cell culture incubator (37°C, 95% humidity, 5% CO2)	Various
Centrifuge (swinging bucket rotor)	Various
Inverse microscope	Various
Liquid nitrogen storage	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).

VEGF165 - 100 µg/mL

Prepare a 100 µg/mL stock solution of VEGF165 according to the manufacturer's instructions. Add to the culture medium to achieve a final concentration of 50 ng/mL (1:2000 dilution).

4.2. Surfaces

Ncyte® Endothelial Cells can be cultured on numerous surfaces.

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

Glass: The attachment of Ncyte® Endothelial Cells on glass surfaces (e.g., cover slips for patch clamp) is not as tight as on plastic ware.

4.3. Coating

Choice of coating depends on the cell and assay purpose. As a standard coating for Ncyte® Endothelial Cell cultivation, we recommend using 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: Use Ncyte® Cardiomyocyte Culture Medium supplemented with Ciprofloxacin and VEGF165 to thaw and maintain the cells.

1. Coat a flask or multi-well plate of desired format with desired coating (see 4.3.)
2. Warm Cardiomyocyte Culture Medium to 37°C.
3. Add 8 mL of Cardiomyocyte Culture Medium 2 to a 50 mL polypropylene tube.
4. Quickly transfer cryopreserved Ncyte® Endothelial Cells from the vapor phase of liquid nitrogen or from a transport dewar with liquid nitrogen directly to a 37 °C water bath.
5. Thaw the vial until the frozen cell suspension detaches from the bottom of the vial and only a small ice clump is visible (~2 minutes).
6. Gently resuspend the sedimented cells by carefully swinging the vial back and forth. Avoid repeatedly pipetting the thawed Ncyte® Endothelial Cells.
7. Transfer the cell suspension to the 50 mL tube using a 1000-µL pipette.

8. Rinse the vial with 1 mL of Cardiomyocyte Culture Medium and add to the cell suspension.
9. Gently mix the cell suspension by carefully inverting the tube. The total volume of cell suspension will now be 10 mL.
10. Pellet Ncyte® Endothelial Cells by centrifugation at 250 x g for 5 minutes at room temperature.
11. Aspirate the supernatant. Gently resuspend the cells in 1 mL of Cardiomyocyte Culture Medium .
12. Count the cell suspension and proceed with cell seeding (see 5.2).

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 Ncyte® Endothelial Cells.

5.2. Seeding

1. Adjust the cell suspension adequately with Cardiomyocyte Culture Medium. The recommended seeding density for cultivation is 45,000 cells/cm² (based on NucleoCounter NC-200®, see Table 4 for recommended volumes). Mix the cells carefully by gently agitating the tube.
2. Remove coating solution from the plates by aspiration; do not let the coating dry.
3. Carefully mix the cells again and plate them into the wells. We recommend using a 12-channel pipette when using 96-well formats. Carefully mix the cells regularly during plating, e.g., after pipetting every 3 rows when using 96-well plates. After plating, make cross-shaped movements to evenly distribute the cells.
4. Incubate cells at 37°C, 5% CO2 in a humidified atmosphere. After placing the cells in the incubator, make again cross-shaped movements to evenly distribute the cells.

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.

5.3. Maintenance

We recommend changing the culture medium every 3-4 days during subsequent culture using Ncyte® Cardiomyocyte Culture Medium supplemented with Ciprofloxacin and VEGF165 at room temperature. Remove 100% of medium and add corresponding amount of fresh medium (Table 4).

6. Appendix

6.1. Morphology in culture

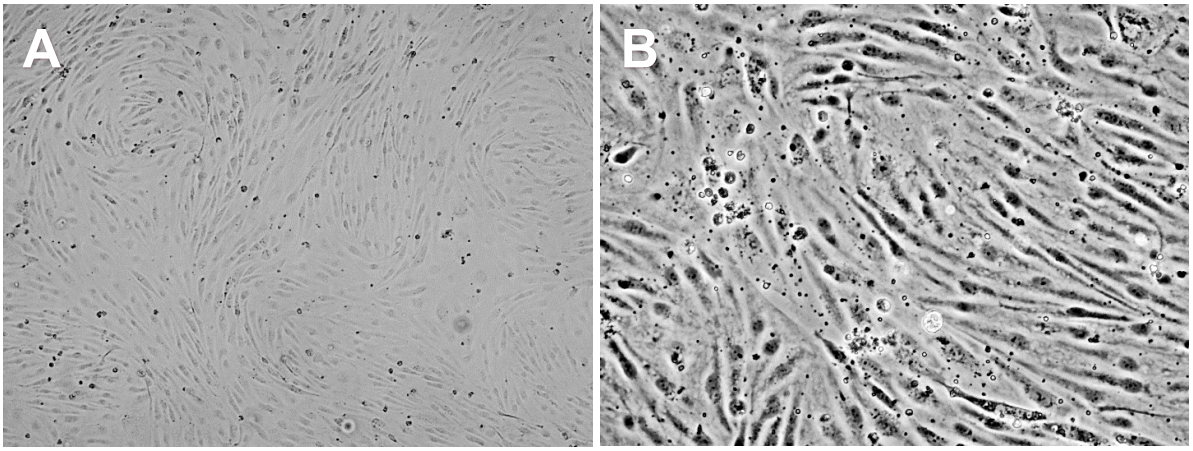

Figure 1: Ncyte® Endothelial Cell morphology 3 days after seeding at 4x (A) and 10x (B) magnification. Cells were seeded at 45,000 cells per cm^2 on a Fibronectin-coated plate.

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