Ncardia
Stem cell experts

Ncyte™ Cortical Neuron Kit

Human iPSC-derived cortical neuron cells

UG-630
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Contents

Getting Started

Please read the entire User Guide carefully prior to thawing or culturing Ncyte™ Cortical Neuron Cells.

The Ncyte Cortical Neuron Kit is for *in vitro* life science research use only.

The Material Safety Data Sheet (MSDS) for the Ncyte Cortical Neuron Kit is available at www.ncardia.com.

Technical Support and Training

Ncardia scientists are available to help. Please direct any questions regarding this User Guide or the Ncyte Cortical Neuron Kit to support@ncardia.com or visit https://ncardia.com/contact-products/ for assistance.

1. General Information

This protocol covers thawing, seeding, and culturing of Ncyte™ Cortical Neuron Cells. Please read the entire protocol prior to starting an experiment. The most recent version of this User Guide can be downloaded from www.ncardia.com.

Ncyte Cortical Neuron 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 in a human skin fibroblast, using a non-viral system.

Ncyte Cortical Neuron Cells are primarily composed of glutamatergic neurons expressing typical markers of the human cerebral cortex neurons, such as Beta III Tubulin, NeuN, MAP2, FOXG1, TBR1 and Ctip2. They exhibit spontaneous activity on microelectrode array (MEA) technology, presenting firing properties as early as Day 4 after thawing and evolve into more complex structured patterns of activity.

2. Safety Information

- The Ncyte™ Cortical Neuron Kit is intended for *in vitro* research use only. The kit is not intended for diagnostics, therapeutic or clinical use and not approved for human *in vivo* applications.
- Ncyte Cortical Neuron 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 Cortical Neuron Cells are inactivated by autoclaving at 121°C for 20 minutes.
- Ncyte Cortical Neuron Cells must 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. Material

3.1 Cells and media provided by Ncardia

Ncyte Cortical Neuron Cells are supplied cryopreserved in a vial containing at least 4 million cells. The complete kit includes cells, medium, and medium supplement (order no. Nc-K-CN).

The Ncyte™ Cortical Neuron Culture medium is prepared by adding Ncyte™ Neuronal Supplement to Ncyte™ Neuronal Basal Medium (see 4.1). Ncyte™ Neuronal Culture medium is used for seeding and culturing of Ncyte Cortical Neuron Cells. Ncyte Neuronal Basal Medium is used for thawing Ncyte Cortical Neuron Cells.

Material	Order number	Container	Content	Storage
Ncyte™ Cortical Neuron Cells	Nc-C-CN	1 cryovial	≥ 4 million cells	Liquid nitrogen
Ncyte™ Neuronal Basal Medium	Nc-M-NBM	1 bottle	250 ml	Liquid, +2-8 °C
Ncyte™ Neuronal Supplement	Nc-MS-NNS	2 cryovials	1.25 ml each	Frozen, -80 °C

Table 1: Overview of Ncyte™ Cortical Neuron Kit II components

Antibiotics can be optionally added to the culture medium. Please note that cell growth and function is not compromised by long-term culture in the presence of Penicillin-Streptomycin.

3.2 Storage conditions

- **Cryopreserved cells:** Upon receipt, transfer the vials directly to the vapor phase of liquid nitrogen for storage. Do not expose the vials to room temperature. Do not store cells at -80 °C, as recrystallization will harm the cells.
- **Medium:** Store Ncyte Neuronal Basal Medium at +2-8°C. Avoid excessive exposure to light.
- For shelf life, see expiry date on bottle. The Ncyte Neuronal Culture medium (Ncyte Neuronal Basal Medium supplemented with Ncyte Neuronal Supplement) can be stored at +2-8 °C for up to 1 week.

3.3 Required consumables

Consumables	Vendor	Cat. No.
Tissue culture flask or multi-well plates	Various	-
Sterile 50 ml polypropylene tubes	Various	-
Sterile Polypropylene bottle	Various	
Poly-L-ornithine (PLO) hydrobromide	Sigma-Aldrich	P3655
Laminin (L) solution	Sigma-Aldrich	L2020
Trypan blue solution 0.4 %	Sigma-Aldrich	T8154
Poly-ethyleneimine solution 50% (w/w) in H_2O	Sigma-Aldrich	P3143
Borate buffer 20x	Sigma-Aldrich	28342

Table 2: Overview of required consumables

3.4 Required equipment

Item	Vendor
Laminar flow hood	Various
Cell culture incubator (37 °C, 95 % humidity, 5 % CO_2)	Various
Fuchs Rosenthal Hemocytometer	Various
Centrifuge (swinging bucket rotor)	Various
Inverse microscope	Various
Liquid nitrogen storage	Various

Table 3: Overview of required equipment

4. Preparation

4.1 Medium

1. If the use of antibiotics is required, add 1.25 mL of a Penicillin-Streptomycin solution (5000 U/mL) to 250 mL Ncyte™ Neuronal Basal Medium for a final concentration of 250 U/mL Penicillin-Streptomycin.
2. Thaw a vial containing Ncyte Neuronal Supplement on ice.
3. Transfer 50 ml Ncyte Neuronal Basal Medium into a polypropylene bottle.
4. Homogenize Ncyte Neuronal Supplement by pipetting; add 1.25 ml into the bottle containing Ncyte Neuronal Basal Medium.
5. Mix by pipetting. Ncyte™ Neuronal Culture medium is ready to use. Store at +2-8°C.

Note

- If <50 ml culture medium is needed, aliquot the Ncyte Neuronal Supplement after thawing and refreeze aliquots. Do not repeatedly thaw and freeze this supplement!
- Do not pre-warm Ncyte Neuronal Basal Medium and Ncyte Neuronal Culture medium to 37 °C to avoid premature degradation of the ingredients.

4.2 Surfaces

Ncyte Cortical Neuron Cells can be cultured on numerous surfaces.

Plastic: Ncyte Cortical Neuron 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 Cortical Neuron Cells on glass surfaces (e.g., cover slips for patch clamp) is not as tight as on plastic ware. Please contact support@ncardia.com for additional information.

4.3 Coating

Choice of coating depends on the cell and assay purpose. As a standard coating for Ncyte™ Cortical Neuron Cell cultivation, we recommend using poly-L-ornithine (PLO)/Laminin (L). For electrophysiological assays, e.g. MEA recordings, we recommend using Polyethyleneimine (PEI) as coating.

Coating plates with poly-L-ornithine (PLO)/Laminin (L)

1. Dilute sterile poly-L-ornithine (PLO) hydrobromide to 10 µg/ml in PBS with Ca^{2+} and Mg^{2+}.
2. Add a proper volume of PLO 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 1 hour.
4. After the incubation period, aspirate PLO and wash 3 times with PBS with Ca^{2+} and Mg^{2+}.
5. Dilute sterile Laminin (L) solution to 10 µg/ml in PBS with Ca^{2+} and Mg^{2+}.
6. Add a proper volume of Laminin solution to cover the bottom of the well.
7. Incubate the culture vessel in a cell culture incubator at 37 °C for 1 hour.
8. Aspirate Laminin immediately before seeding.
   Note: Do not allow the surface to dry during PLO/Lamin coating.
9. If the culture vessel is not used immediately, it can be stored at 37 °C for a maximum of 24 hours.

Coating with poly-ethyleneimine (PEI)

Please contact support@ncardia.com for information about coating with PEI or refer to the application protocol when available.

Format	Volume coating (ml)	Volume media (ml)
T75 flask	8	15
T25 flask	2.5	5
48-well plate Maestro MEA	0.2	0.4
96-well plate	0.05	0.05

Table 4: Suggested coating and media volumes per culturing format

5. Cell Culture

5.1 Thawing

Note: Use Ncyte™ Neuronal Basal Medium (without supplement) for thawing of cells.

1. Coat a flask or multi-well plate of desired format with desired coating (see 4.3.)
2. Warm Ncyte Neuronal Basal Medium to room temperature.
3. Add 1 ml Neuronal Basal Medium to a 50 mL polypropylene tube.
4. Quickly transfer cryopreserved Ncyte Cortical Neuron 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 neuronal cells.
7. Transfer the cell suspension to the 50-mL tube using a 1000-µL pipette.
8. Rinse the vial with 1 mL of Ncyte Neuronal Basal Medium and add to the cell suspension.
9. Gently mix the cell suspension by carefully swinging the tube back and forth.
   Optional: Transfer 20 µL of the cell suspension to a 1.5 mL tube for cell counting before centrifugation.
10. Add 7 mL Ncyte Neuronal Basal Medium to the cell suspension in the 50-ml tube and gently mix the cell suspension to achieve a total volume of 10 mL.
11. Pellet Ncyte Cortical Neuron Cells by centrifugation at 500 x g for 8 minutes at room temperature.
12. Aspirate the supernatant. Gently resuspend the cells in 1 mL Ncyte Cortical Neuronal Culture medium.
13. Use 20 µL of cell suspension for cell counting (see 5.2). Proceed with cell seeding (see 5.3).

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 neuronal cells.

5.2 Counting of cells and determination of viability

Note: We highly recommend to perform cell counting manually using a hemocytometer. For example, by using the Fuchs Rosenthal Counting Chamber (figure 1).

1. Add 20 µl trypan blue solution to 20 µL cell suspension withdrawn for counting. Mix carefully.
2. Apply 20 µl of the 1:1 mixture to the counting chamber and count viable (clear), dead (blue) and total cells.
3. Count the total number of cells according to Equation 1.

Count four #2 squares according to Figure 1.
Viable cells: __ + __ + __ + __ = __ (#vc)
Non-viable (blue) cells: __ + __ + __ + __ = (#nvc)
([#vc] / 4) x 2 x 5000 = __ cells / mL
(# of cells / mL) x (total volume from step 5.1.10) = cells in total
Viability = (#vc) / ([#vc] + [#nvc] x 100) = __%

Equation 1: Cell counting

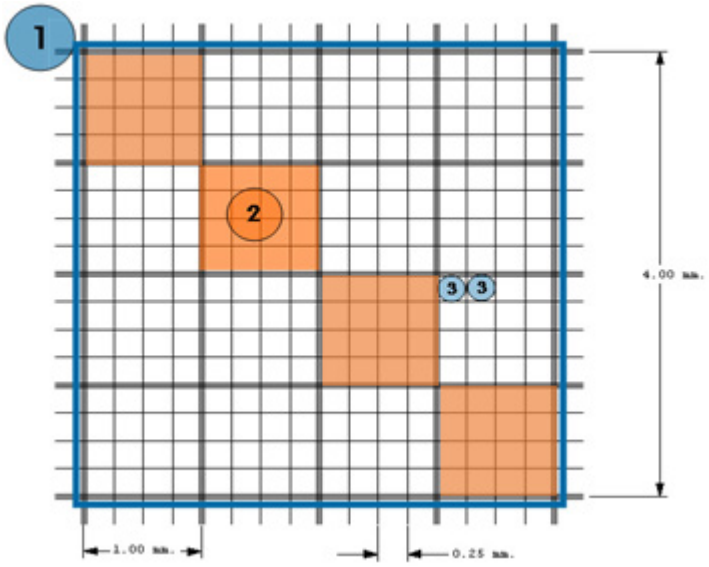

Fig. 1: Layout of a Fuchs Rosenthal Counting chamber

5.3 Seeding

1. Adjust the cell suspension adequately with culture medium depending on the suggested assay conditions (see Table 5). Mix the cells carefully by gently agitating the tube.
2. Transfer the coated plates and the cell suspension to the laminar flow hood.
3. Remove coating solution from the plates by aspiration; do not let the coating dry.
4. Carefully mix the cells again and plate them into the wells. We recommend using a 8-channel pipette when using 96-well plates. Carefully mix the cells regularly during plating, e.g., after pipetting 3 rows each when using 96-well plates.
5. Leave plates for 30 minutes under the hood to achieve an even distribution of the cells in the wells. Incubate cells at 37 °C, 5 % CO_2 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.

Assay and format	No. of cells per surface area	Volume of cell suspension
Immunostaining (96-well format)	150,000/cm²	200 µL/well
Maestro MEA (96-, 48-well format)	80,000/well	Droplet, 10 µL/well

Table 5: Overview of seeding densities according to assay type

5.4 Maintenance

We recommend changing the culture medium 3 times a week during subsequent culture using Ncyte Cortical Neuronal Culture medium at room temperature. Remove 50 % of the medium and add a corresponding amount of fresh medium. Repeat this process once.

Note: Cells are very sensitive to complete medium changes (100 %), which is why partial (50 %) medium changes are recommended.

6. Appendix

6.1 Ncyte™ Cortical Neuron Cell morphology in culture

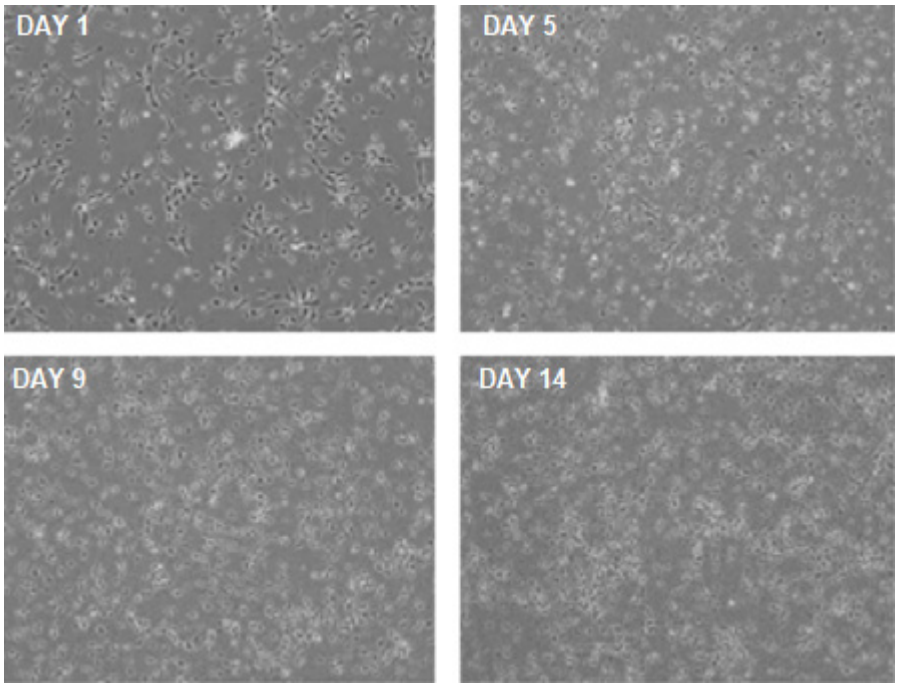

Fig. 2: Ncyte Cortical Neuron Cell morphology at different times after seeding. Cells were seeded at 150,000 cells per cm^2 on a Poly-L-ornithine/Laminin coated plate. Magnification 200x.

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