Ncardia
Stem cell experts

Ncyte™ Astrocyte Kit II

Human iPSC-derived astrocytes

UG-605
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Contents

Getting started

Please make sure to read the entire User Guide carefully before you start thawing and culturing using this product.

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

Technical support

Scientific experts are ready to assist with any questions regarding this User Guide or the Ncyte Astrocyte Kit.

Visit www.ncardia.com for more information or send an email request to support@ncardia.com.

1. General information

This protocol covers thawing, seeding, and culturing of Ncyte Astrocyte cells. Please read the entire protocol before using this product.

Ncyte Astrocyte cells are produced through a well-defined *in vitro* differentiation process from human induced pluripotent stem cells (hiPSC). They are a highly enriched culture of astrocytes that are used in cell-based *in vitro* assays in co-culture with neurons for pharmacology and toxicology.

2. Safety information

- The Ncyte Astrocyte Kit II is 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 Astrocyte 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 Astrocyte cells can be inactivated by autoclaving at 121°C for 20 minutes.
- Ncyte Astrocyte cells should be cultured in a sterile environment.

It is highly recommended to wear gloves and lab coats while handling all reagents. Please consult the Certificate of Analysis (CoA) and material safety data sheets (MSDS) for additional safety instructions where applicable.

3. Material

3.1 Cells and media provided

Ncyte Astrocyte cells are supplied cryopreserved in a vial containing 2 million cells. The Ncyte Astrocyte Kit II includes cells, medium and medium supplement. The kit contains components for 100 ml of culture medium, which is sufficient for approximately 3 weeks of cultivation.

Astrocyte Culture Medium is prepared by adding the Ncyte Astrocyte Supplement (provided) to Ncyte Neuronal Basal Medium (provided). It is used for seeding and culturing of Ncyte Astrocyte cells. Ncyte Neuronal Basal Medium is used for thawing of the cells.

Material	Container	Content	Storage
Ncyte Astrocyte cells	1 cryo vial	>2 million cells	Liquid nitrogen
Ncyte Neuronal Basal Medium	1 bottle	250 ml	Liquid, +4°C
Ncyte Neuronal Supplement	2 cryo vials	1.25 ml each	Frozen, -80°C

Table 1: Overview of Ncyte Astrocyte Kit II components.

Antibiotics may be added to the culture medium if desired. Note that antibiotic use is optional.

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 Ncyte Neuro Basal Medium at +4°C. Avoid excessive exposure to light. For storage life, see expiry date on bottle. Ncyte Neuronal Basal Medium with added Ncyte Neuronal Supplement can be stored at 4°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	-
Poly-L-ornithine (PLO) hydrobromide	Sigma-Aldrich	P3655
Laminin (L) solution	Sigma-Aldrich	L2020
Matrigel® hESC-Qualified Matrix	Corning	354277
Trypan blue solution 0.4 %	Sigma-Aldrich	T8154

Table 2: Overview of required consumables

3.4 Required equipment

Equipment
37°C water bath
Laminar flow hood
Cell culture incubator (37°C, 95 % humidity, 5 % CO_2)
Neubauer hemocytometer
Centrifuge (swinging bucket rotor)
Inverse microscope
Liquid nitrogen storage

Table 3: Overview of required equipment

4. Preparations

4.1 Medium

1. Thaw a vial containing Ncyte Neuronal Supplement on ice.
2. Transfer 50 ml Ncyte Neuronal Basal Medium to a 50 ml polypropylene tube.
3. Homogenize Ncyte Neuronal Supplement by gently pipetting up and down. Pipet 1.25 ml to the Ncyte Neuronal Basal Medium in the 50 ml tube.
4. Mix by pipetting the tube contents. The resulting Astrocyte Culture Medium is ready to use. Store at 4°C.

Note

- If less than 50 ml culture medium is needed, aliquot the Ncyte Neuronal Supplement after thawing and refreeze in portions. Do not repeatedly thaw and freeze the supplement.
- Do not pre-warm Ncyte Neuronal Basal Medium or Astrocyte Culture Medium to 37°C, to avoid premature degradation of the ingredients.

4.2 Surfaces

Ncyte Astrocyte cells can be cultured on various surfaces.

Plastic

Ncyte Astrocyte cells adhere best to cell culture-treated plastic surfaces. It is recommended to use plasticware from Nunc™ (Nunclon Delta Surface) or Greiner (Greiner Bio-One).

Glass

The attachment of Ncyte Astrocyte cells on glass surfaces (e.g., cover slips for patch clamp) is not as tight as on plasticware.

4.3 Coating

Choice of coating depends on the intended assay. As a standard coating for cultivation of Ncyte Astrocyte cells, it is recommended to use poly-L-ornithine (PLO)/Laminin (L) or Matrigel.

For electrophysiological assays in mixed cultures with neurons, e.g. MEA recordings, it is recommended to use a Polyethyleneimine (PEI) coating.

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

Note: Do not allow the surface to dry during coating.

1. Dilute sterile poly-L-ornithine (PLO) hydrobromide to 10 µg/ml in PBS without 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 h.

4. After the incubation period, aspirate PLO and wash 3 times with PBS without Ca^{2+} and Mg^{2+}.
5. Dilute sterile Laminin (L) solution to 10 µg/ml in PBS without Ca^{2+} and Mg^{2+}.
6. Add enough 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.

Coating with Matrigel

Handle Matrigel® hESC-Qualified Matrix in accordance with the manufacturer's instructions. Keep Matrigel and dilutions of Matrigel on ice during all steps and use pre-cooled tubes, pipettes, plates, and dishes. Matrigel will rapidly solidify at room temperature.

1. Thaw Matrigel on ice overnight at 4°C.
2. Dilute Matrigel 1:100 with ice-cold Ncyte Neuronal Basal Medium.
3. Add diluted Matrigel to plates to be coated. The volume should be sufficient to cover the entire surface (see table 4).
4. Incubate at room temperature for 1 hour.
5. Plates not used immediately may be wrapped in parafilm and stored at 4°C for up to 2 weeks.
6. Before plating astrocytes, warm up the plates for 1 hour at room temperature.
7. Aspirate Matrigel immediately before seeding the astrocytes.

Format	Volume coating (ml)	Volume media (ml)
T75 flask	10	15
T25 flask	5	5
24-well plate	0.5	1
48-well plate	0.3	0.5
96-well plate	0.05	0.1

Table 4: Suggested coating and media volumes per culturing format

5. Cell Culture

5.1 Thawing

Note: Use Ncyte Neuronal Basal Medium (without supplements) 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 9 ml Ncyte Neuronal Basal Medium to a 50 ml polypropylene tube.
4. **Optional:** If cells should be counted prior to centrifugation, add 1 ml Ncyte Neuronal Basal Medium to a 50 ml polypropylene tube and thaw cells according to steps 5 to 8 described below. Withdraw a 20 µl aliquot for counting, and immediately add 8 ml of Ncyte Neuronal Basal Medium to make a total volume of 9 ml.

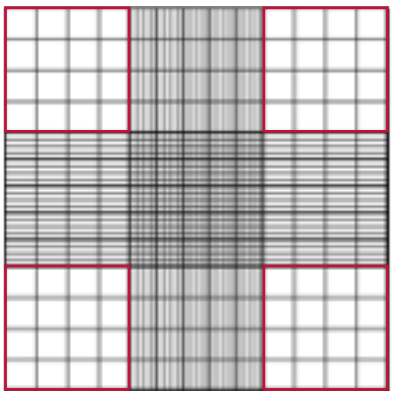
5. Quickly transfer cryopreserved Ncyte Astrocyte cells from the vapor phase of liquid nitrogen or from a transport dewar with liquid nitrogen directly to a 37°C water bath.
6. Thaw the vial until the frozen cell suspension detaches from the bottom of the vial and only a small ice clump is visible (2 minutes).
7. Gently resuspend the sedimented cells by gently flicking the bottom of the vial. Avoid repeated pipetting of the thawed astrocytes.
8. Carefully transfer the cell suspension to the 50 ml tube using a 1000 µl pipette tip.
9. Gently mix the cell suspension by gently shaking the 50 ml tube. The total volume of the cell suspension will now be 10 ml.
10. Pellet Ncyte Astrocyte cells by centrifugation at 500 x g for 8 min at room temperature.
12. Aspirate the supernatant. Gently resuspend the cells in 1 ml Astrocyte Culture Medium.
13. Use 20 µl of cell suspension for cell counting (see 5.2) and proceed with cell seeding (see 5.3).

Note

- For transport 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 the transport as this might affect cell viability.
- Avoid damage of the thawed astrocytes by repeated pipetting, vigorous shaking or vortexing. Careful mixing of the cells is key to ensuring maximal viability.

5.2 Counting of cells and determination of viability

1. Add 20 µl trypan blue solution to 20 µl cell suspension withdrawn for counting.
2. Apply 10 µl of the 1:1 mixture to a Neubauer hemocytometer and count viable (clear), dead (blue) and total cells.
3. Count the number of cells in each of the four outer squares highlighted in red of Figure 1. Calculate the mean number of cells per red square.
4. Calculate the number of cells corrected by chamber factor (1 x 10^4), dilution factor (2), and total volume of cell solution (e.g., 1 ml).



Calculation example

Mean number of viable cells per square = 100

100 x 10,000 x 2 x 1 = 2,000,000

2 million living cells in the cell suspension

Figure 1: Neubauer hemocytometer

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 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 formats. Carefully mix the cells regularly during plating, e.g., after pipetting every 3 rows 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.

Format	No. of cells per surface	Volume of cell suspension
24-well	40,000/cm²	1000 µl/well
48-well	40,000/cm²	500 µl/well
96-well	40,000/cm²	100 µl/well

Table 5: Overview of seeding densities for Ncyte Astrocyte cells.

5.4 Maintenance

We recommend changing the culture medium 2-3 times a week during subsequent culture using Astrocyte Culture Medium at room temperature. Remove 100 % of medium and add corresponding amount of fresh medium. Repeat this process once.

Ncyte Astrocyte cell cultures are viable for more than 3 weeks.

6. Appendix

6.1 Morphology in culture

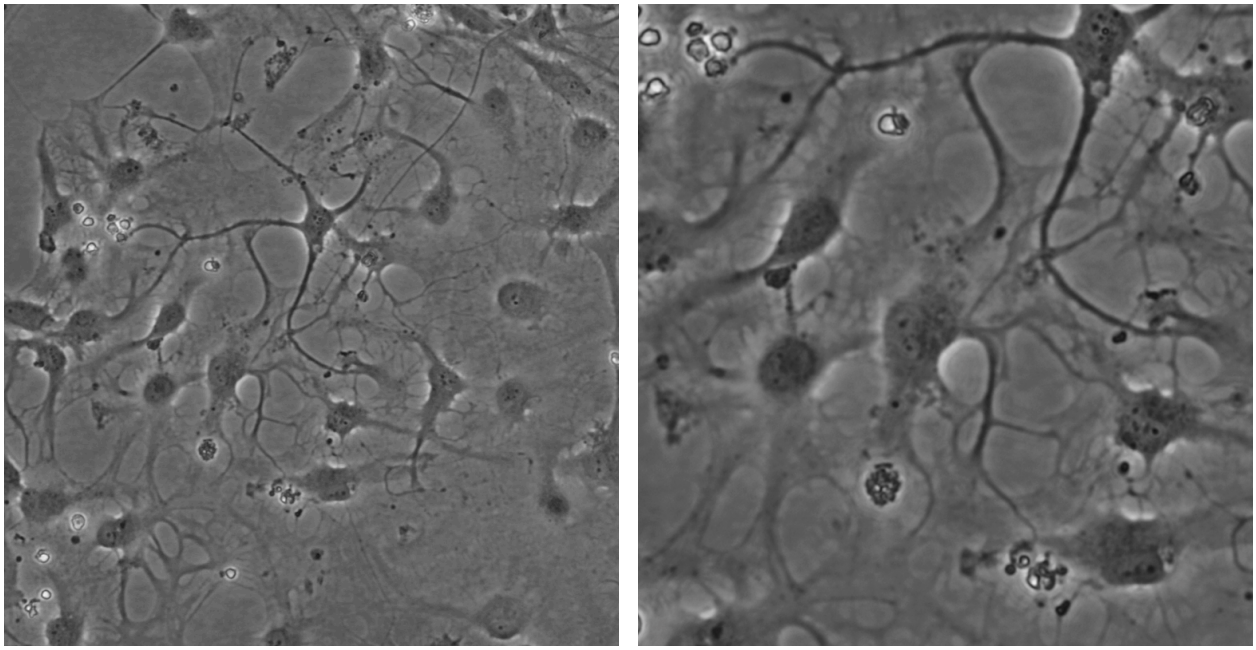


Fig. 2: Ncyte Astrocyte cell morphology 7 days after seeding. Cells were seeded at 40K cells per cm^2 on a Matrigel-coated plate. Medium was changed every 2 to 3 days. Magnification (a) 200x (b) digital magnification of (a).

6.2 Immunostaining

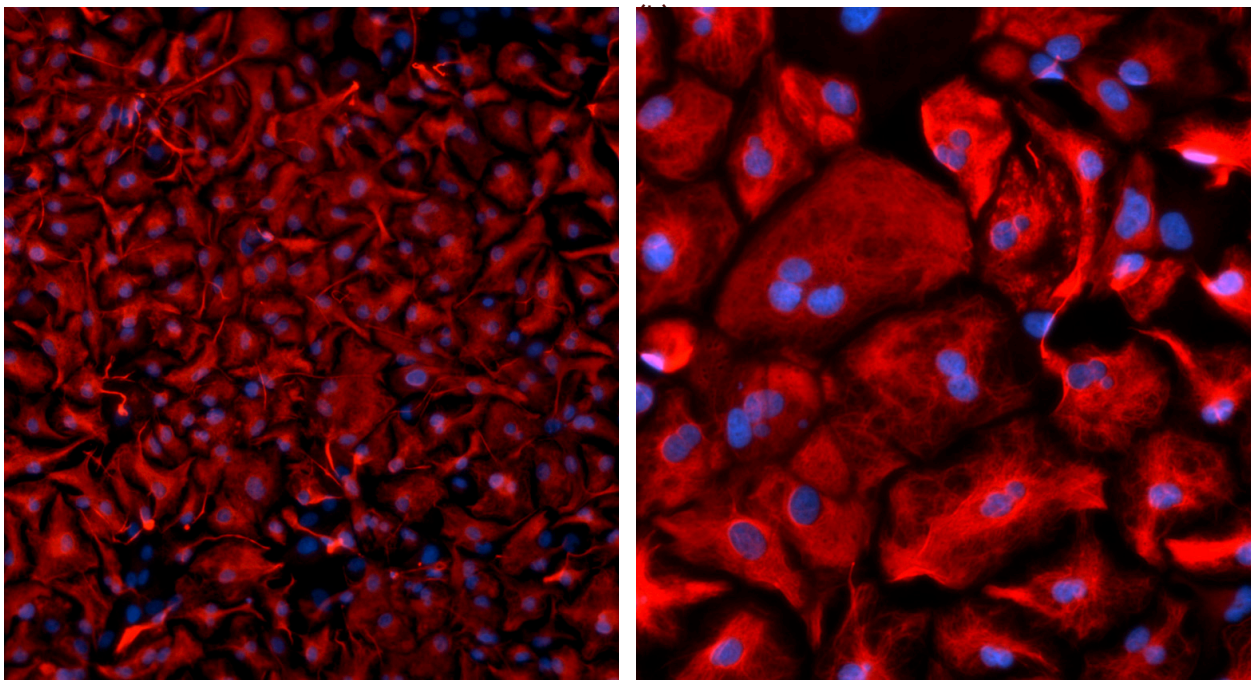


Fig. 3: Immunostaining of Ncyte Astrocyte cells 7 days after thawing. Astrocytes (GFAP) in red and nucleus (DAPI) in blue. Magnification 200x.

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