Maintenance of Novus Human Cerebral Cortical Neurons (Frozen Young hCCNs)

Instruction Manual

Version 3.0

Powered by Axol Bioscience Ltd.

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NOVUS CELL REAGENTS

Specially formulated cell supplements to complement our neural progenitor cells and cortical neurons

NBP2-31344

Neural Maintenance Media
Provided as 500ml/kit (4 bottles of 125mL)
with 7.5ml supplement

NBP2-31350

Neural Coating Solution (orange color cap)
Provided as 50X stock solution (3 x 120uL)

NBP2-31351

Neural Advance™ (red color cap)
Provided as 1000X stock solution (4 x 26uL)

NBP2-31352

Sure Mix™ (purple color cap)
Provided as 1000X stock solution (3 x 26uL)

NBP2-31353

Neural Unlock™ (white color cap)
Provided as 1000X stock solution (1 x 25uL)

NBP2-31355

Sure Boost™ (yellow color cap)
Provided as 1000X stock solution (1 x 10uL)

NBP2-31356

Neural Enhance™ (green color cap)
Provided as 1000X stock solution (1 x 15uL)

NBP2-31357

Neural Sure Growth™ (blue color cap)
Provided as 1000X stock solution (1 x 15uL)

NOVUS
a bio-techne brand

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Preparing Complete Novus Neural Maintenance Medium

1. Thaw **Novus Neural Maintenance Medium Supplement** (7.5 mL) overnight at 4°C.

2. Add the entire 7.5mL of the thawed **Novus Neural Maintenance Medium Supplement (NBP2-31344)** to the entire 500 mL **Novus Neural Maintenance Medium**. Mix well.

3. Pre-warm the **Complete Novus Neural Maintenance Medium** to 37°C before use.

Top Tip: We recommend you store your **Complete Novus Neural Maintenance Medium** (**NBP2-31344)** in 50 mL aliquots and pre-warming each aliquot before use.

Preparing Matrix for Adherent Cell Culture

1. Thaw the **Novus Neural Coating Solution (NBP2-31350) overnight at 4°C**.
2. Check the total number of viable cells on the cryovial or on the Certificate of Analysis shipped with the cells.

3. Calculate the total surface area that requires coating. This is the total number of viable cells (e.g. 2 million) / your desired plating density (e.g. no less then 50,000 cells/cm^2).

4. Dilute the **Novus Neural Coating Solution** stock solution **(50X)** in D-PBS (without calcium or magnesium) to make **1X working solution e.g. 120 µL in 6 mL**.
5. Coat the surface of your culture vessel with the **Novus Neural Coating Solution 1X working solution**. We recommend coating at a concentration of 100 **µL per cm^2**, however, please optimize for your experiments.

6. Incubate your culture vessel **overnight at 37ºC**.

Warning: Do make sure that coating doesn't evaporate during the night. Do not wash the vessel after coating with Novus Neural Coating Solution or let the Novus Neural Coating Solution coating dry out before applying the cells. Ensure the culture vessel is evenly coated, if not there will be varied attachment efficiency.

Thawing Novus hyCCNs

1. Remove the cells from dry ice or liquid nitrogen storage. Immediately transfer the cells to a **37°C water bath.**

2. Quickly thaw the vial of cells by swirling it in the **37°C water bath**. Do not completely submerge the vial. Remove the vial before the last bit of ice has melted.

3. When thawed, immediately transfer the cells into a 15 mL sterile conical tube, and carefully add **10 mL** of pre-warmed **Complete Novus Neural Nurture Medium**.

4. Centrifuge the cells at **200 g for 5 mins**, and discard the supernatant.

5. While centrifuging cells make **Complete Novus Neural Maintenance Medium** supplemented with **Novus Sure Boost™** 1000X stock solution to make it 1X (**i.e. 1 µL Novus Sure Boost™ per mL medium**).

6. Resuspend the cell pellet in **Complete Novus Neural Maintenance Medium** supplemented with **Novus Sure Boost™**.

7. Remove the **Novus Neural Coating Solution** coating solution from the overnight pre-coated culture vessel before plating resuspended cells.

8. Plate the resuspended cells at **no less than 50,000 cells/cm^2** on your **Novus Neural Coating Solution** coated culture vessel.

9. Incubate the plated cells at **37°C, 5% CO_2.**

Top Tip: Make sure that you distribute the cells evenly by slightly tilting the culture vessel back and forth. This will promote consistent cell density, monolayer and health throughout the culture and help to avoid edge effects and variations in cellular maturity. In addition after seeding, avoid disturbing the culture vessel for a minimum of 30 minutes to allow the cells to adjust to their environment.

Maintaining Novus hyCCNs

1. The morning after plating the hyCCNs, replace the medium with fresh Complete Novus Neural Maintenance Medium supplemented with **Novus Neural Enhance™** (**1000X stock solution** i.e. 1 µL **Novus Neural Enhance™** per mL medium), and **Novus Sure Mix™** (**500X stock solution** i.e. 2 µL **Novus Sure Mix™** per mL medium

2. Culture the cells in the supplemented medium for **two days**, then switch to the **Complete Neural Maintenance Medium without Novus Neural Enhance™** and **Novus Sure Mix™**

3. Exchange medium every other day. Supplement the **Complete Neural Maintenance Medium** with **Novus Sure Mix™** only once every 4 days e.g. with every other medium change as this prevents clumping of the neuronal cell bodies.

Top Tip: We recommend that you culture these cells for 40+ days after thawing before proceeding to analysis of synaptic marker expression or electrophysiology experiements.

Characterising the Differentiated Novus hyCCNs

- To monitor synaptic marker expression or conduct electrophysiology experiments we recommend culturing the cells for 35 days after induction of differentiation.

- ICC analysis of marker expression is a simple way for characterising cell identity. Please refer to Novus's guide to ICC, which can be found at:

  http://www.novusbio.com/support/support-by-application.html

- See Figure 1 for representative images of ICC staining of hCCN-neuronal cultures.

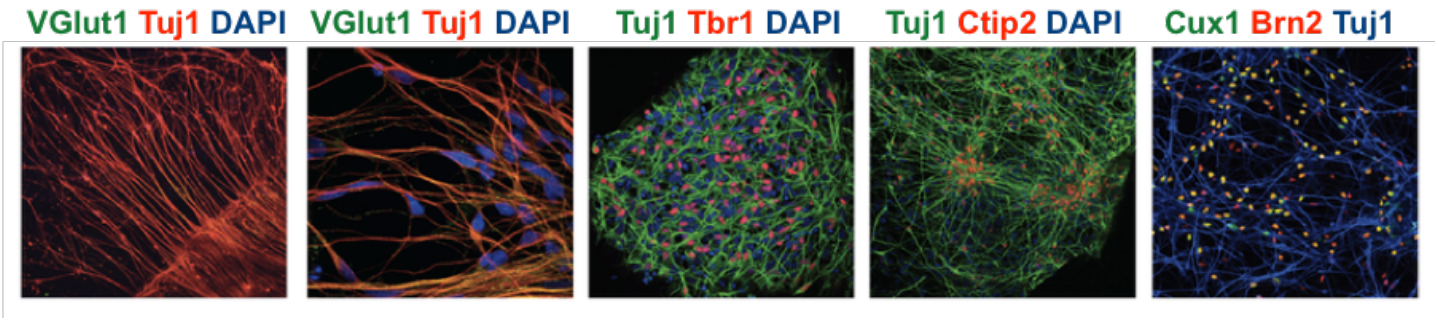


Figure 1: Representative Images of the differentiated Novus hyCCNs.

Technical Support

- ## Online Resources

  Please visit our website at http://www.novusbio.com/ for additional product information and *Support*, including instruction manuals and application protocols.

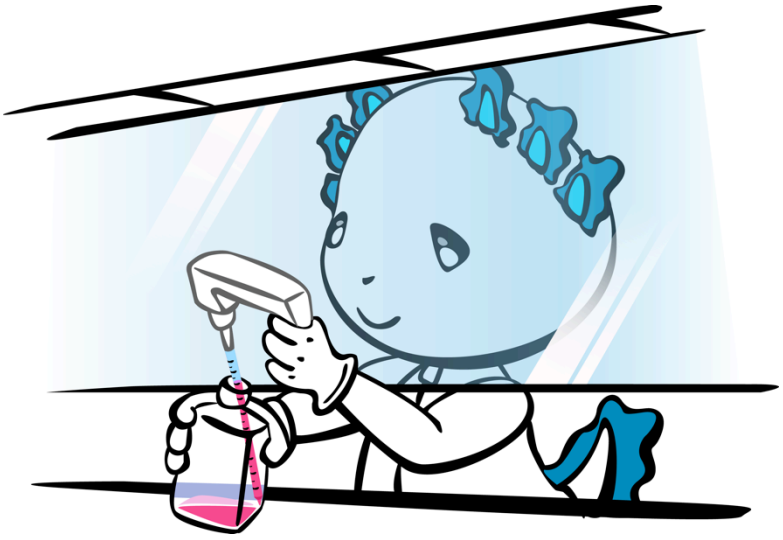
- ## Contact Us

  For more information or technical assistance, call 303-730-1950, or email technical@novusbio.com. US Toll Free Tel: 1-888-506-6887.

- ## Certificate of Analysis

  The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis can be requested from our Technical Support team.

Notes



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