Culture and Differentiation of Novus Human Neural Progenitor Cells (hNPCs)

Instruction Manual

Version 3.0

NOVUS
a bio-techne brand

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)

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www.novusbio.com• info@novusbio.com • P: 303.730.1950

Notes

Preparing Complete Novus Neural Maintenance Medium

1. Thaw Novus Neural Maintenance Medium Supplement (NBP2-31344 provided as 7.5ml supplement) overnight at 4°C.
2. Using sterile technique add the entire 7.5mL of the thawed Novus Neural Maintenance Medium Supplement (NBP2-31344) to the entire 500 mL Novus Neural Maintenance Basal 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** **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. Using a sterile technique 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** dry out before applying the cells.

Thawing Novus hNPCs

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 and gently transfer the cells using 1ml pipette into a 15 mL sterile conical tube, and carefully add **10 mL** of pre-warmed **Complete Novus Neural Maintenance Medium**.

4. Immediately centrifuge the cells at **200 g for 5 mins (in order to minimize time that cells are sitting in freezing medium)**, 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 of Novus Sure Boost™ mL of medium**).

6. Resuspend the cell pellet in **Complete Novus Neural Maintenance Medium** supplemented with **Novus Sure Boost™** by first carefully dislodge the pellet by squirting 1mL of your **Novus Sure Boost™** supplemented medium using a 1 mL tip.
7. When cells are resuspened remove the **Novus Neural Coating Solution** from the 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** for **two hours.**

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 formation 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.

10. **Two hours** after plating, replace the medium with fresh, pre-warmed **Complete Novus Neural Maintenance Medium** supplemented with **Novus Sure Growth™** 1000X stock solution to make it 1X (**i.e. 1 µL Novus Sure Growth™ per mL medium**).

11. After two days, replace the medium with fresh, pre-warmed **Complete Novus Neural Maintenance Medium** without **Novus Sure Growth™**. Refresh the medium every other day.

12. When the culture is between **70-80%** confluent, your culture is ready to undergo synchronised differentiation or passaging (See **Figure 1**)

Passaging of Novus hNPCs

1. When the hNPCs reach about 80% confluence they are ready to be passaged. Make sure to pre-coat the surface of your culture vessel used for passaging with the **Novus Neural Coating Solution** (**NBP2-31350**) **1X working solution day before**.
2. Thaw **Novus Neural Unlock™ (NBP2-31353)** directly before use and store at **4°C.**
3. Following day pre-warm the **Complete Novus Neural Maintenance Medium** (**NBP2-31344**) to **37°C**.
4. Discard the spent medium from the culture vessel.
5. Gently rinse the surface of the cell layer once with the D-PBS (without calcium or magnesium, **2 mL D-PBS per 10 cm^2 culture surface area**).
6. Discard the D-PBS.
7. To detach the cells, add **1 mL of cold Novus Neural Unlock™ (NBP2-31353) per 10cm^2 culture surface area**. Evenly distribute it over the whole cell layer. Incubate the cells for **5 minutes at 37°C by returning it to the incubator.**
8. Transfer cells to conical tube and stop the cell dissociation reaction by adding **four volumes** of **Complete Novus Neural Maintenance Medium** (**NBP2-31344)** (e.g. if 1 mL of **Novus Neural Unlock™** (**NBP2-31353**) is used, then add 4 mL of the Complete Medium to stop the reaction). Pipette up and down a few times to disperse the medium.

9. Centrifuge the tube at **200 g for 5 minutes**. Discard the supernatant.

10. Resuspend the cell pellet in 4 mL of pre-warmed **Complete Novus Neural Maintenance Medium** (**NBP2-31344**), and take a sample to determine the total number of viable cells.

11. Remove the diluted **Novus Neural Coating Solution** from the pre-coated culture vessel.

12. Add enough cell suspension to the coated vessel to provide **60,000 cells per cm^2**. Ensure an even plating of the hNPCs by gentle rocking the culture vessel back and forth and side-to-side several times.

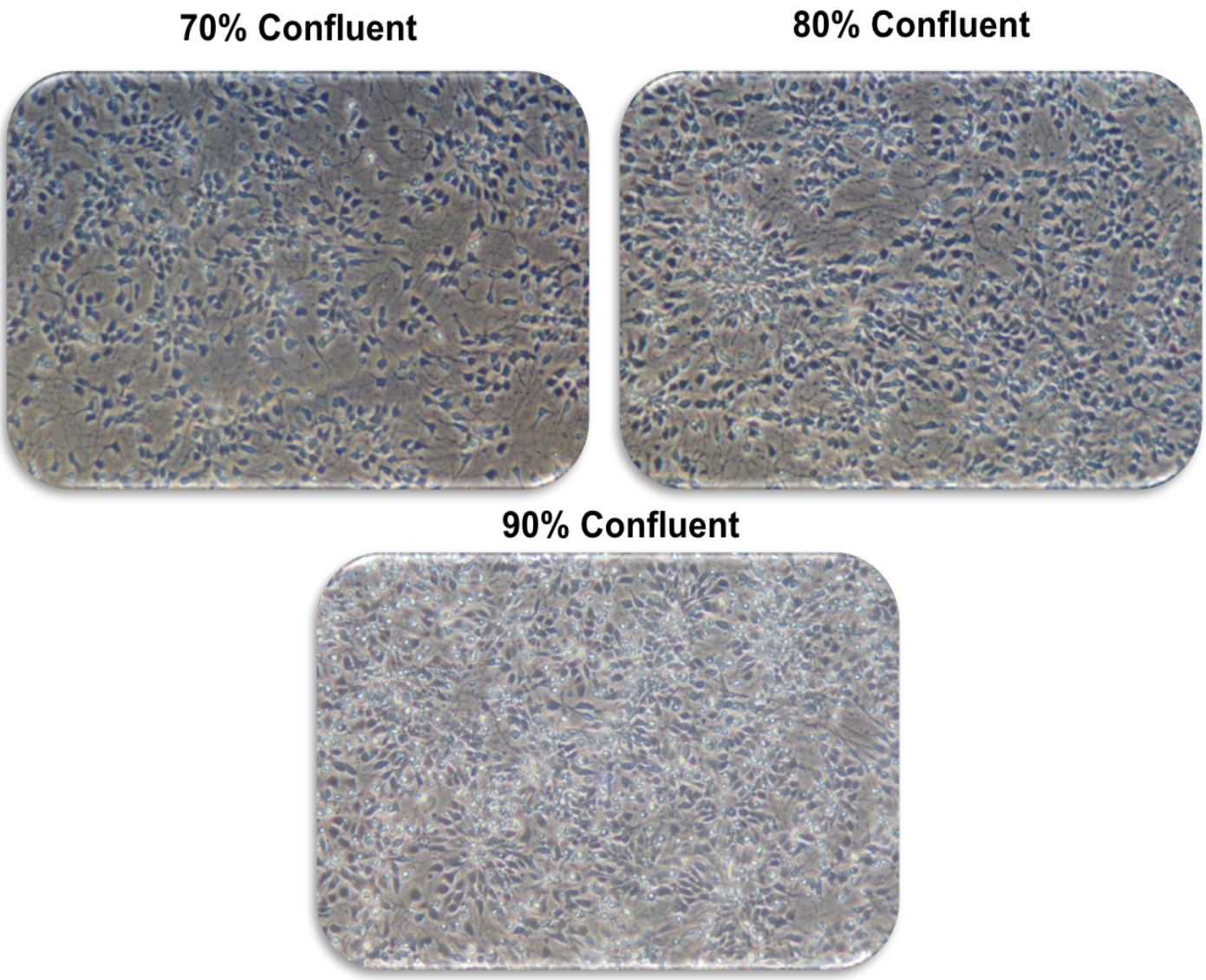


Figure 1: Representative images of Novus hNPC cultures at 70%, 80% and 90% confluency.

13. Incubate the cells at **37°C, 5% CO_2.** Re-feed the culture with fresh **Complete Novus Neural Maintenance Medium** (**NBP2-31344**) every other day.

14. **Every 4 days** (i.e. every other medium change) supplement the **Complete Novus Neural Maintenance Medium** with **Novus Sure Mix™** (**NBP2-31352, 500X stock solution) to make 1X dilution** i.e. 2 µL per mL medium, as this prevents clumping of the neuronal cell bodies**.**

Top Tip: For consistent results in your differentiation studies and other experiments, we recommend using cells **below passage 2**.

Differentiation of Novus hNPCs

- **To initiate spontaneous differentiation of the hNPCs into neurons and astrocytes:**

1. Plate Novus hNPCs on an **NovusNeural Coating Solution** coated, tissue culture-treated plate at no less then **50,000 cells per cm^2** following the protocol for passaging the hNPCs (page 8).

2. Replace the medium with fresh **Complete Novus Neural Maintenance Medium (NBP2-31344)** **every other day**.

3. Supplement the medium with **Novus Sure Mix™** (**NBP2-31352, 500X stock solution** i.e. 2 µL per mL medium) **once every 4 days**. This prevents clumping of the neuronal cell bodies**.**

- **To induce synchronized differentiation:**

1. Plate Novus hNPCs on an **Novus Neural Coating Solution** coated, tissue culture-treated plate at no less then **50,000 cells per cm^2** following the protocol for passaging the hNPCs (page 8).

2. When the cells reach 70-80% confluence, replace the medium with fresh **Complete Novus Neural Maintenance Medium (NBP2-31344)** supplemented with **Novus Neural Advance™** (**NBP2-31351, 1000X stock solution** i.e. 1µL per mL medium) and **Novus Sure Mix™** (**NBP2-31352, 500X stock solution** i.e. 2 µL per mL medium)

3. Incubate the cells at **37°C, 5% CO_2** for **two days**, then switch to **Complete Novus Neural Maintenance Medium** (**NBP2-31344).**

4. Re-feed the culture with fresh medium every other day. Supplement the **Complete Novus Neural Maintenance Medium** (**NBP2-31344)** with **Novus Sure Mix™** (**500X**) **once every 4 days**. This prevents clumping of the neuronal cell bodies**.**

Characterising Novus hNPCs and Their Differentiated Progeny

- To harvest cells for transplantation, we recommend following the method for passaging cells (page 8) up to point 9 and then resuspending the cells in your transplantation medium at the desired density.

- 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 characterizing 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 **Figures 2 & 3** for representative images of ICC staining of hNPC cultures pre- and post-differentiation.

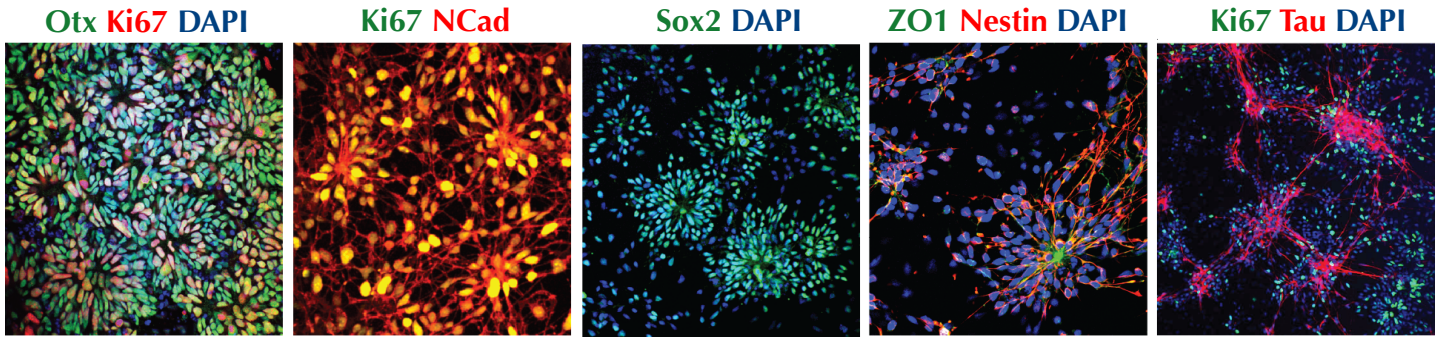


Figure 2: Representative Images of Novus hNPCs

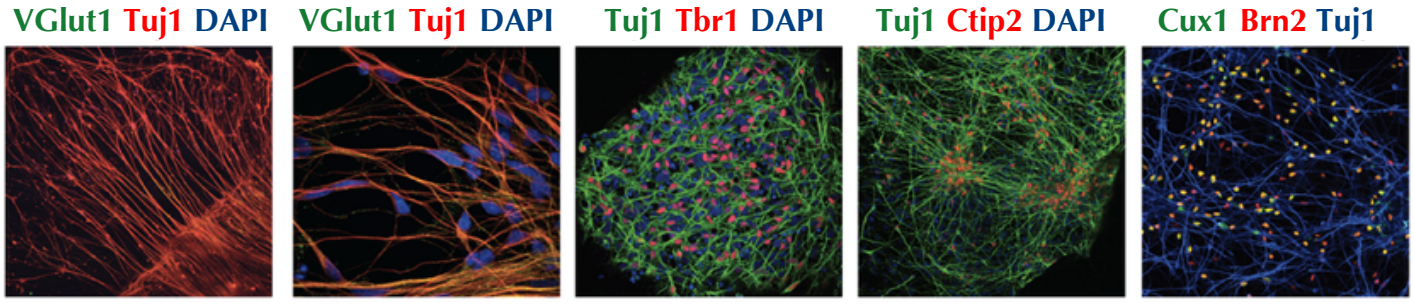


Figure 3: Representative Images of the differentiated progeny of Novus hNPCs after synchronised differentiation period using Neural Differentiation Medium.

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.

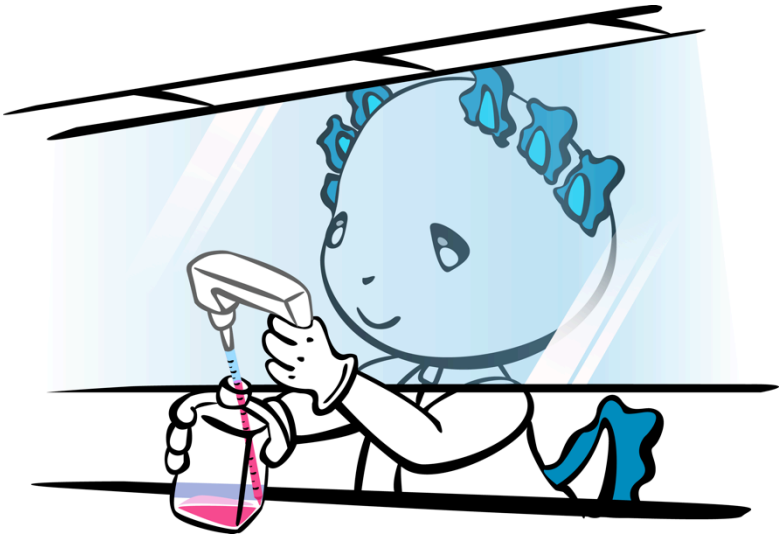
- ## Contact Us

  For more information or technical assistance, call 303-730-1950,
  fax 303-730-1966, 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



Don’t forget to rate, review and register your Novus product at http://www.novusbio.com/