

Maintenance of Axol™ Human Cerebral Cortical Neurons (hCCNs Plated)

Xeno-Free System

Instruction Manual

Version 5.0

XF Protocol - 8

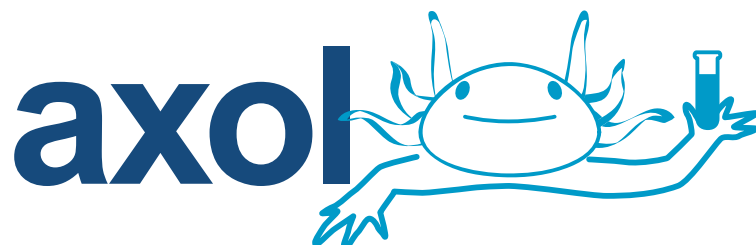


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Product Information

Catalog no.	Product Name:	Format	Stock Concentration.	Storage on Arrival:	Thawing Instructions:	Storage Once Thawed:
ax0025 - ax0026	Axol hCCNs	6M viable cells per plate	NA	37°C	NA	NA
ax0032-500	Axol Neural Maintenance-XF Medium	1 x 500 mL	NA	Aliquot and store at -80°C for up to 6 months. Keep in dark	Overnight at 4°C	Once, thawed, store aliquot at 4-8°C for up to 1 week

Preparation of Neural Maintenance-XF Medium

1. Upon receipt aliquot and store your **Axol Neural Maintenance-XF Medium** at or below **-20°C** protected from light. Stored at **-20°C**, media is stable for 6 months from date of manufacture.
2. When ready to use, thaw an aliquot of media overnight at **4°C** in the dark.
3. A thawed, supplemented aliquot of **Axol Neural Maintenance-XF Medium** can be stored at **4°C** for 1 week. Protect from light.
4. Pre-warm the **Axol Neural Maintenance-XF Medium** to **37°C** before use.

Top Tip: We recommend you store your **Axol Neural Maintenance-XF Medium** in 50 mL aliquots at **-20°C**. Pre-warm each aliquot to **37°C** before use.

Maintaining Axol hCCNs

1. Open the package and place the 96-well plate into a class II biosafety cabinet. Make sure you spray the plate with 70% ethanol, and wipe it down with tissue.
2. Remove the silicone sealing cap on the plate.
3. Place the plate into a **37°C, 5% CO₂** incubator and incubate for **2 hours**.
4. Aspirate the medium from the wells and replace with **200 µL pre-warmed** the **Axol Neural Maintenance-XF Medium** per well of the 96-well plate. (we recommend storing your medium in 50 mL aliquots and pre-warming each aliquot to **37°C** for 15 minutes as needed).
5. Re-feed the culture with half the volume of spent medium with fresh, pre-warmed **Axol Neural Maintenance-XF Medium** every four days.

Top Tip: We recommend that you culture these cells for 35 days after receipt before proceeding to analysis of synaptic marker expression or electrophysiology experiments.

Characterising Axol hCCNs

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

<http://www.axolbio.com/application-protocols.html>

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

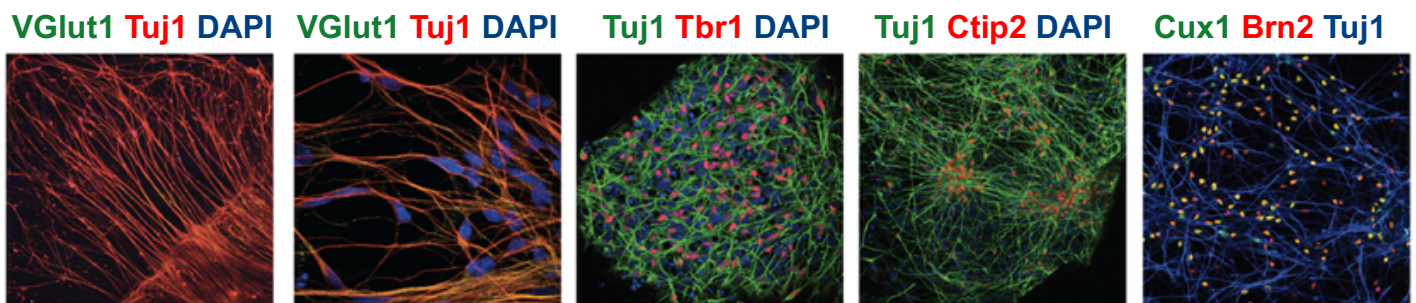


Figure 1: Representative Images of the differentiated progeny of Axol hCCNs.

The following list includes some of the primary antibodies that can be used for characterizing Axol hCCNs:

Tuj1
Neuronal Marker (axon), Abcam
ab14545

MAP2
Neuronal Marker (dendrite), Novus
NB300-213

Doublecortin
Newborn Neuron, Abcam ab18723

Tbr1
Deep-layer Cortical Neurons,
Abcam ab31940

Brn2
Upper-layer Cortical Neurons,
Santa Cruz sc-6029

VGlut1
Glutamatergic Neurons, Synaptic
Systems 135303

S100
Astrocytes, Dako Z0311

PSD-95
Postsynaptic Terminals, Abcam
ab2723

Synaptophysin
Presynaptic Terminals, Abcam
ab68851

Technical Support

- Online Resources

Please visit our website at www.axolbio.com for additional product information and *Technical Resources*, including instruction manuals, application protocols, video guides, wall charts and webinars.

- Contact Us

For more information or technical assistance, call +44 (0)1223 751051, or email support@axolbio.com. US Toll Free Tel: 1-800-678-AXOL (2965) US Toll Free Fax: 1-800-861-2965 (1-800-861-AXOL).

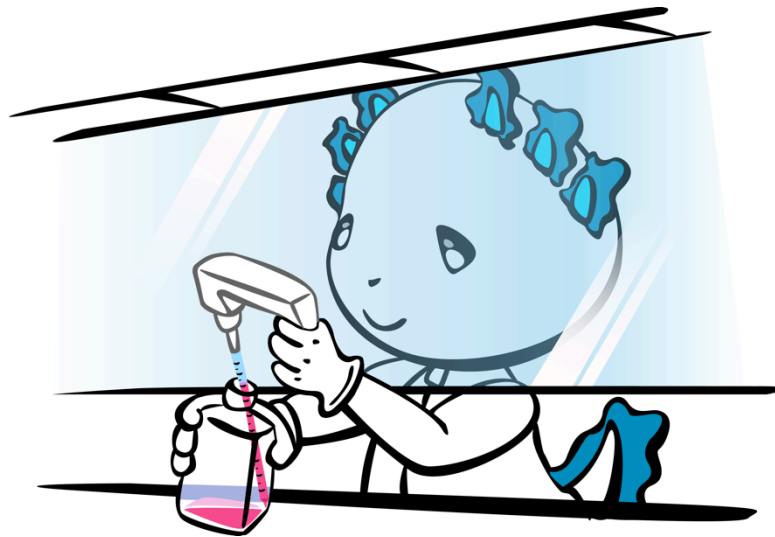
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- Certificate of Analysis

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Go to www.axolbio.com/certificate-of-analysis-lookup and search for the Certificate of Analysis with product lot number, which is printed on the cryovial label.



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