Differentiation of Axol Human Neural Progenitor Cells (NSCs) into Cerebral Cortical Neurons

With Expansion Step

Xeno-Free System

Instruction Manual

Version 2

XF Protocol - 3

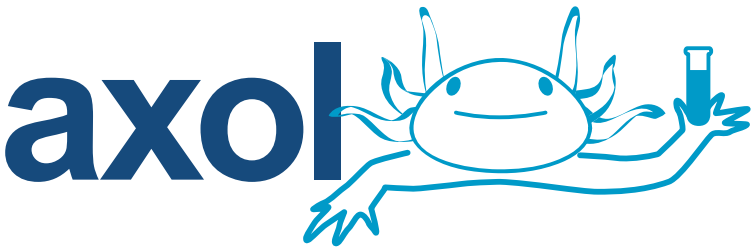
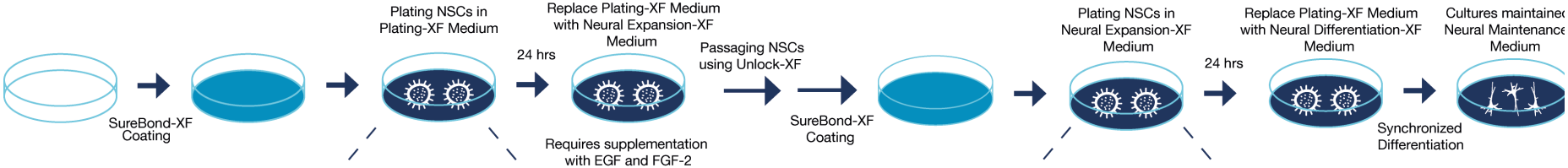


Table of Contents

Product Information

Catalog no.	Product Name	Format	Stock Concentration	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0030-500	Axol Neural Expansion-XF Medium	1 x 500 mL	1X	Aliquot and store at - 80°C for up to 6 months. Keep in dark	Overnight at 4ºC	Once, thawed, store aliquot at 4-8°C for up to 1 week
ax0034-125	Axol Neural Differentiation-XF Medium	1 x 125 mL	1X	Aliquot and store at - 80°C for up to 6 months. Keep in dark	Overnight at 4ºC	Once, thawed, store aliquot at 4-8°C for up to 1 week
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 4ºC	Once, thawed, store aliquot at 4-8°C for up to 1 week
ax0041XF	Axol SureBondXF	1x 1 mL	200X	4°C	N/A	Store at 4-8°C for up to 1 month
ax0044-XF	Axol Unlock-XF	1 x 100 mL	1X	Aliquot and store at - 80°C for up to 5 months	Overnight at 4ºC	Once, thawed, store aliquot at 4-8°C for up to 1 week
ax0033	Axol Plating-XF Medium	1 x 30 mL	1X	- 20°C	Overnight at 4ºC	Must be used immediately once thawed
ax0047X-100µg	Axol Sure GrowthX, Recombinant Human EGF	1x 100 µg lyophilized powder	N/A	- 20°C	N/A	For long term storage, the powder should be reconstituted in a phosphate-buffered saline to a concentration of no less than 100µg/ml. It is recommended to add a carrier protein (i.e. 0.1% HSA). Reconstituted protein should be used immediately or stored in working aliquots at - 20°C
ax0047-100µg	Axol Sure Growth, Recombinant Human FGF_2 (FGF basic 146)	1x 100 µg lyophilized powder	N/A	- 20°C	N/A	For long term storage, the powder should be reconstituted in a phosphate-buffered saline to a concentration of no less than 100µg/ml. It is recommended to add a carrier protein (i.e. 0.1% HSA). Reconstituted protein should be used immediately or stored in working aliquots at - 20°C store at 4-8°C for up to 1 week

Fully-defined system to differentiate NSCs into neurons



Density Guidelines: Protocol 3

Catalog Number	Background	Minimum Density	Maximum Density
ax0015	Healthy	50,000 cells per cm²	200,000 cells per cm²
ax0016	Healthy	50,000 cells per cm²	200,000 cells per cm²
ax0111	Alzheimer's Disease (ApoE4 homozygote)	70,000 cells per cm²	200,000 cells per cm²
ax0112	Alzheimer's Disease (PSEN-1 L286V)	70,000 cells per cm²	200,000 cells per cm²
ax0113	Alzheimer's Disease (PSEN-1 M146L)	70,000 cells per cm²	200,000 cells per cm²
ax0114	Alzheimer's Disease (PSEN-1 A246E)	70,000 cells per cm²	200,000 cells per cm²
ax0115	Alzheimer's Disease (PSEN-2 N141L)	70,000 cells per cm²	200,000 cells per cm²
ax0211	Huntington's Disease (CAG:45)	70,000 cells per cm²	200,000 cells per cm²

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ax0211	Huntington's Disease (CAG:45)	60,000 cells per cm²	200,000 cells per cm²

Preparation of Plating-XF Medium

1. Upon receipt store your **Axol Plating-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 plating media overnight at **4°C** in the dark.

3. Once thawed, **Axol Plating-XF Medium** should be used immediately and **should not be** used for subsequent experiments.

Preparation of Neural Expansion-XF Medium

1. Upon receipt aliquot and store your **Axol Neural Expansion-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. Prepare a **100µg/mL** solution **(5000X)** of **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)** and **Axol Sure GrowthX, Recombinant Human EGF** by resuspending the 100µg of lyophilized powder in 1 mL of PBS supplemented with 0.1% HSA.

4. Supplement the **Axol Neural Expansion-XF Medium** with **100µg/mL (5000X) Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)** and **100µg/mL (5000X) Axol Sure GrowthX, Recombinant Human EGF** to a final concentration of **20 ng/mL (1X)** of FGF2 and EGF.

5. A thawed, supplemented aliquot of **Axol Neural Expansion-XF Medium** can be stored at **4°C** for 1 week. Protect from light.

Preparation of Neural Differentiation-XF Medium

1. Upon receipt, aliquot and store your **Axol Neural Differentiation-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 Differentiation-XF Medium** can be stored at **4°C** for 1 week. Protect from light.

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.

Preparation of Unlock-XF

1. **Axol Unlock-XF** comes as a complete 1X solution.

2. Upon receipt, aliquot and store your **Axol Unlock-XF** at or below **-20°C**. Stored at **-20°C**, solution is stable for 6 months from date of manufacture.

3. When ready to use, thaw an aliquot overnight at **4°C**. A thawed aliquot of **Axol Unlock-XF** can be stored at **4°C** for 1 month.

Preparing Matrix for Adherent Cell Culture (ax0041XF)

1. Check the total number of viable cells on the cryovial or on the Certificate of Analysis shipped with the cells.

2. 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. 50,000 cells/cm^2).

3. Dilute the **Axol SureBondXF** stock solution **(200X)** in D-PBS (without calcium or magnesium) to make **1X working solution** **e.g. 30 µL in 6 mL**.

4. Coat the surface of your culture vessel with the **Axol SureBondXF** **1X working solution**. We recommend coating at **200 µL 1X solution per cm^2**

5. Incubate for 4 hours at 37ºC.

Warning: Do not wash the vessel after coating with Axol SureBondXF.

Do not allow Axol SureBondXF -coated culture vessels to dry.

Thawing Axol NSCs

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 **Axol Plating-XF Medium.**

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

5. Resuspend the cell pellet in the required amount of **Axol Plating-XF Medium**. We recommend the use of **200 µl Axol Plating-XF Medium per cm^2**.

6. Quickly remove the diluted **Axol SureBondXF** coating solution from the pre-coated culture vessel before plating resuspended cells.

7. Plate the resuspended cells with **Axol Plating-XF Medium** according to density guidelines (see Page 4) on your **Axol SureBondXF** coated culture vessel.

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

9. **24 hours** after plating, replace the plating medium with fresh **Axol Neural Expansion-XF** supplemented with **Axol Sure GrowthX, Recombinant Human EGF** and **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)**.

Top Tip: It is critical to promote consistent cell density, monolayer and health throughout the culture to avoid edge effects and variations in cellular maturity. After seeding, avoid disturbing the culture vessel for a minimum of 30 minutes to allow the cells to adjust to their environment.

1. After two days, replace the medium with fresh, **Axol Neural Expansion-XF Medium** supplemented with **Axol Sure GrowthX, Recombinant Human EGF** and **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)**.

2. Re-feed the culture with fresh **Axol Neural Expansion-XF** supplemented with **Axol Sure GrowthX, Recombinant Human EGF** and **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)** every two days.

3. When the culture is between **70-80%** confluent, passage your Axol NSCs as indicated in the following section.

Passaging of Axol NSCs for Differentiation

1. When the NSCs reach about 80% confluence they are ready to be passaged. Make sure to pre-coat the surface of your final culture vessel with **Axol SureBondXF** prior to passaging.

2. Thaw an aliquot of **Axol Unlock-XF** directly before use and pre-warm at **37°C.**

3. Discard the spent medium from the culture vessel.

4. 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**).

5. Discard the D-PBS.

6. To detach the cells, add **1 mL of pre-warmed Axol Unlock-XF per 10cm^2 culture surface area**. Evenly distribute it over the whole cell layer. Incubate the cells for **3 minutes at 37°C by returning it to the incubator.**

7. Transfer an equal volume of pre-warmed **Axol Neural Expansion-XF Medium** supplemented with **Axol Sure GrowthX, Recombinant Human EGF** and **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)** to the culture vessel. For example, if 1 mL of **Axol Unlock-XF** is used, then add 1 mL of the Medium. This will stop the cell dissociation reaction

8. Gently flush the cells off the culture vessel being careful not to create bubbles. Take off diluted media and place into conical tube. Top up the conical tube with an equal volume of **Axol Neural Expansion-XF Medium** supplemented with **Axol Sure GrowthX, Recombinant Human EGF** and **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)**. If 6 mL of diluted media in conical tube, add 6 mL of Medium). 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 1 mL of complete **Axol Neural Expansion-XF Medium** supplemented with **Axol Sure GrowthX, Recombinant Human EGF** and **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)**. Take a sample to determine the total number of viable cells.

11. In a sterile 50 mL conical tube, add the required volume of cells needed for your applications (see Page 4 for density guidelines). Add **Axol Neural Expansion-XF**

Medium supplemented with **Axol Sure GrowthX, Recombinant Human EGF** and **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)** to dilute your cell solution to the appropriate volume. The overall volume should be sufficient to plate cells at **200 µL per cm^2** for your chosen tissue culture vessel.

12. Quickly remove the diluted **Axol SureBondXF** coating solution from the pre-coated culture vessel ensuring the vessel does not dry.

13. Add the cell suspension to the coated vessel. Ensure an even plating of the NSCs by gently rocking the culture vessel back and forth and side-to-side several times.

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

15. **2 hours** after plating, replace the medium with fresh, pre-warmed **Axol Neural Expansion-XF** supplemented with **Axol Sure GrowthX, Recombinant Human EGF** and **Axol Sure Growth, Recombinant Human FGF2 (FGF basic 146)**.

16. **24 hours** after plating, replace the spend medium with fresh pre-warmed **Axol Neural Differentation-XF Medium**.

17. After **72 hours**, replace half the volume of spent medium with fresh, **Axol Neural Maintenance-XF Medium**.

18. After **24 hours**, replace half the volume of spent medium with fresh, pre-warmed **Axol Neural Maintenance-XF Medium**. Repeat this process of re-feeding cultures with half-volumes of fresh, pre-warmed **Axol Neural Maintenance-XF Medium** every four days.

Top Tip: Cultures can be maintained under these conditions for over 50 days in culture!

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 497 119, or email support@axolbio.com. US Toll Free Tel: 1-800-678-2965 (1-800-678-AXOL), US Toll Free Fax: 1-800-861-2965 (1-800-861-AXOL).

  Where we are:
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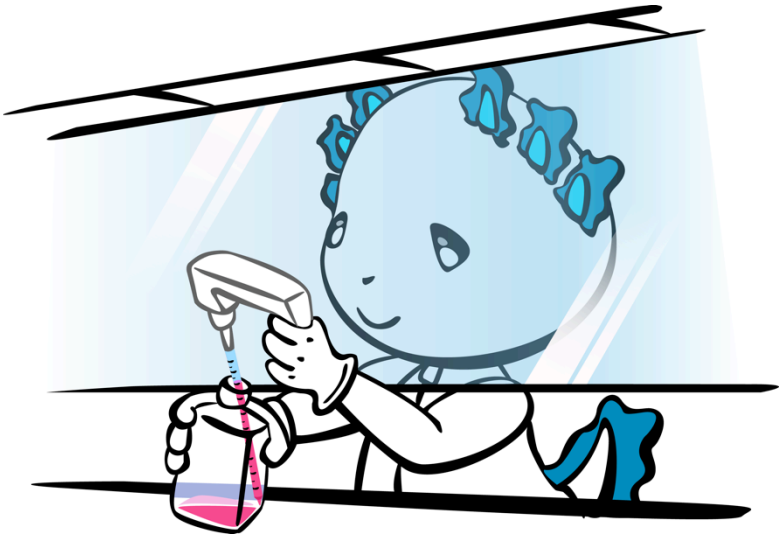
- ## Certificate of Analysis

  The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website.

  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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