Maintenance of Axol NSCs in 96 well plating format

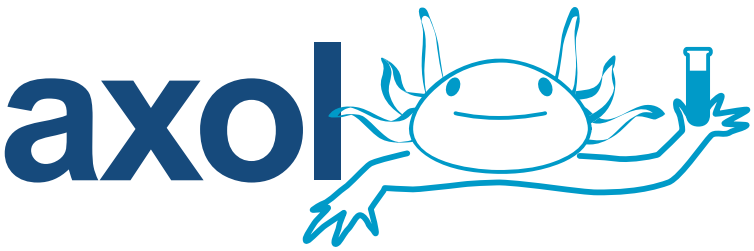
For densities >50000/cm^2

Xeno-free system

Instruction Manual

Version 1.5

XF Protocol - 5



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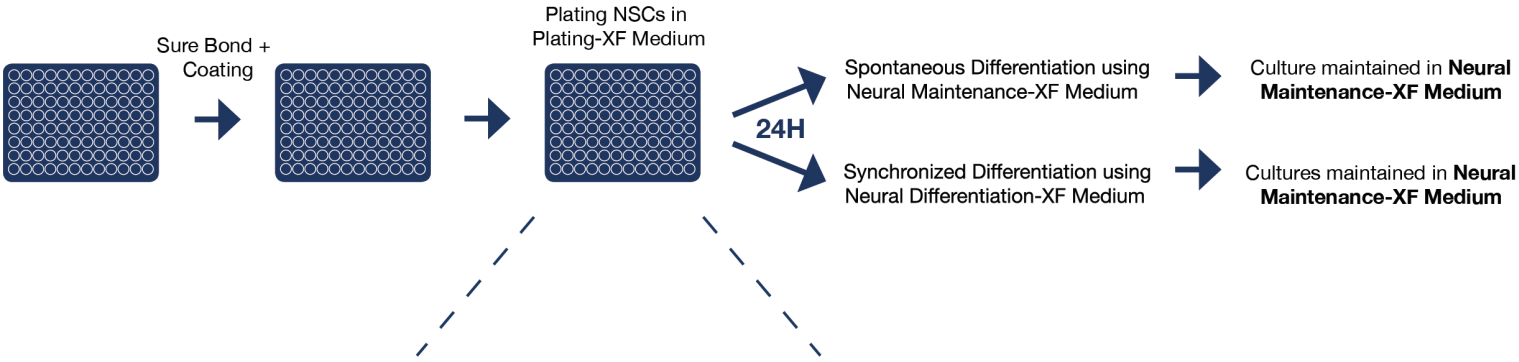
Product Information

Catalog no.	Product Name	Format	Stock Concentration	Storage on Arrival	Thawing Instructions:	Storage Once Thawed:
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
ax0033	Axol Plating-XF Medium	1 x 30 mL	1X	- 20°C	Overnight at 4ºC	Must be used immediately once thawed

Fully-defined system to culture NSCs in 96 well format

Protocol 5: Speciality Formats

96 well plate; densities above 50,000/cm²



Density Guidelines: Protocol 5

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

Preparation of Plating-XF Medium

1. Upon receipt, store **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 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** can be stored at **4°C** for 1 week. Protect from light.

Preparation of Neural Maintenance-XF

1. Upon receipt, the user should aliquot and store **Axol Neural Maintenance-XF** 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.

Preparing Matrix for Adherent Cell Culture Using Axol SureBondXF (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 (follow density guidelines on Page 4).

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 **470 µL 1X solution per cm^2. In 96 well format, this equate to 150µl/well.**

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.

   Please count cells to ensure optimal seeding density.

5. Resuspend the cell pellet in **Axol Plating-XF Medium**.

6. Quickly remove the diluted **Axol SureBondXF** coating solution from the pre-coated culture vessel before plating resuspended cells. **It is recommended you leave behind 30% (45 µl) of the Axol SureBondXF to ensure the wells do not dry.**

7. Plate the resuspended cells at **no less than 50,000 cells/cm^2 (**see density guidelines on Page 4**)** on your **Axol SureBondXF** coated 96 well plate at 120 µl per well. In total, the wells will contain 165 µl.

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

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.

Spontaneous Differentiation of Axol NSCs

1. **24 hours** after plating, replace 115 µl spent medium with 100 µl of fresh, pre-warmed **Axol Neural Maintenance-XF Medium** per well.

2. Re-feed the culture with half the volume of spent medium (75 µl) with fresh, pre-warmed **Axol Neural Maintenance-XF Medium** (75 µl) every four days.

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

OR

Synchronous Differentiation of Axol NSCs

1. **24 hours** after plating, replace 115 µl spent medium with 100 µl of fresh, pre-warmed **Axol Neural Differentiation-XF Medium** per well.

2. After **72 hours,** re-feed the culture with half the volume of spent medium (75 µl) with fresh, pre-warmed **Axol Neural Maintenance-XF Medium** (75 µl).

3. **24 hours after last media change,** replace 75 µl spent medium with 75 µl of fresh, pre-warmed **Axol Neural Maintenance-XF Medium** per well.

4. Re-feed the culture with half the volume of spent medium (75 µl) with fresh, pre-warmed **Axol Neural Maintenance-XF Medium** (75 µl) 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.

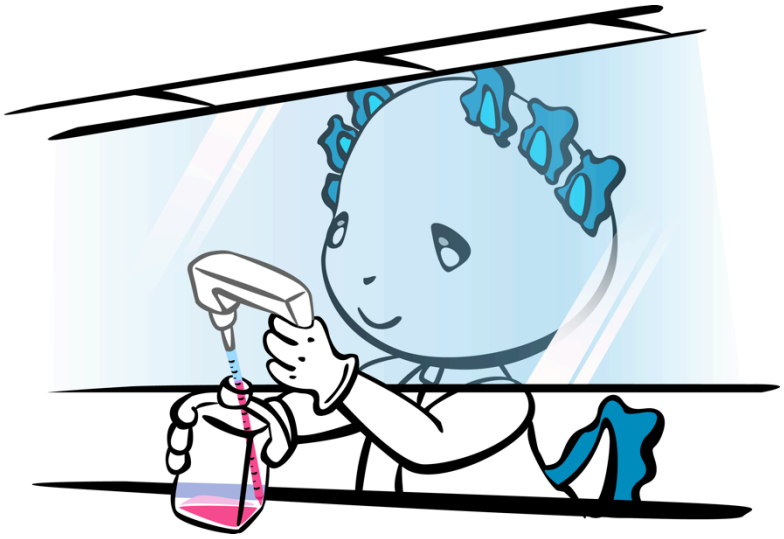
- ## Contact Us

  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).

- ## 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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