

Maintenance of Axol NSCs in 96 well plating format

For densities $50000/cm^2$

Instruction Manual

Version 1.5

XF Protocol - 6

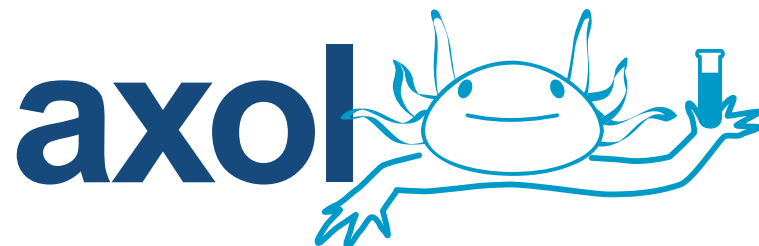


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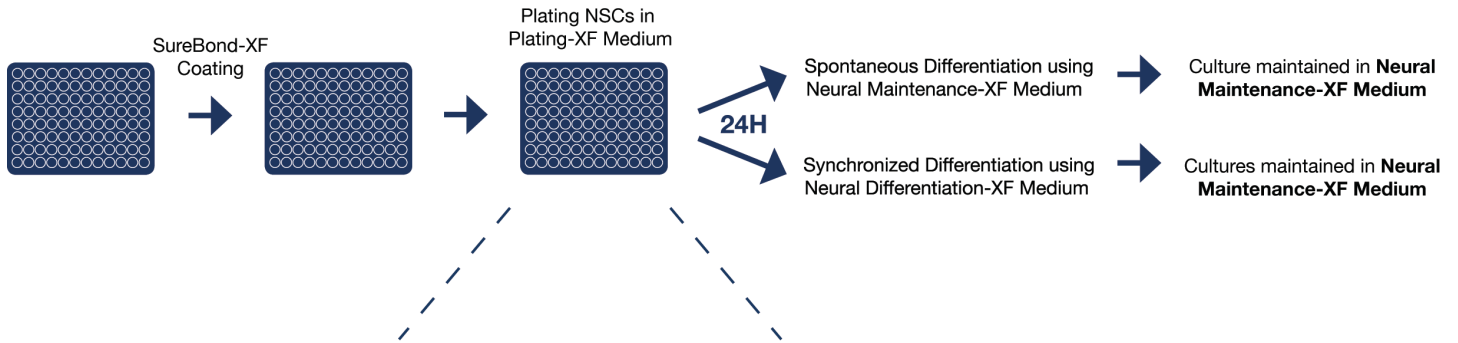
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Product Information

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 4°C	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 4°C	Once, thawed, store aliquot at 4-8°C for up to 1 week
ax0041+	Axol Sure Bond+ (Includes Axol Sure Bond™ / Axol ReadySet Solution)	3 x 120 µL 1 x 10 mL	50X 1X	- 80°C RT	Overnight at 4°C NA	Store at 4-8°C for up to 2 weeks Store at 4-8°C for up to 1 month
ax0033	Axol Plating-XF Medium	1 x 30 mL	1X	- 20°C	Overnight at 4°C	Must be used immediately once thawed

System to culture NSCs in 96 well format at low density

96 well plate; low density



Density Guidelines: Protocol 6

Catalog Number	Background	Minimum Density	Maximum Density
ax0015	Healthy	10,000 cells per cm ²	200,000 cells per cm ²
ax0016	Healthy	10,000 cells per cm ²	200,000 cells per cm ²
ax0111	Alzheimer's Disease (ApoE4 homozygote)	Spontaneous Differentiation: 70,000 cells per cm ² Synchronous Differentiation: 70,000 cells per cm ²	200,000 cells per cm ²
ax0112	Alzheimer's Disease (PSEN-1 L286V)	Spontaneous Differentiation: 30,000 cells per cm ² Synchronous Differentiation: 70,000 cells per cm ²	200,000 cells per cm ²
ax0113	Alzheimer's Disease (PSEN-1 M146L)	Spontaneous Differentiation: 30,000 cells per cm ² Synchronous Differentiation: 70,000 cells per cm ²	200,000 cells per cm ²
ax0114	Alzheimer's Disease (PSEN-1 A246E)	Spontaneous Differentiation: 30,000 cells per cm ² Synchronous Differentiation: 70,000 cells per cm ²	200,000 cells per cm ²
ax0115	Alzheimer's Disease (PSEN-2 N141L)	Spontaneous Differentiation: 30,000 cells per cm ² Synchronous Differentiation: 70,000 cells per cm ²	200,000 cells per cm ²
ax0115	Alzheimer's Disease (PSEN-2 N141L)	Spontaneous Differentiation: 30,000 cells per cm ² Synchronous Differentiation: 70,000 cells per cm ²	200,000 cells per cm ²
ax0211	Huntington's Disease (CAG:45)	Spontaneous Differentiation: 30,000 cells per cm ² Synchronous Differentiation: 70,000 cells per cm ²	200,000 cells per cm ²

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.

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 SureBond+ (ax0041+)

1. Calculate the total surface area that requires coating. This is the total number of viable cells (e.g. 2 million) / your desired plating density. **Axol Sure Bond+** can support low density cultures to a minimum of 10,000 cells/cm² (**Dependent on cell line. Please refer to density guidelines**). Please check the cell count provided on the COA.
2. Thaw the **Axol Sure Bond** coating solution **overnight at 4°C**.
1. Pre-coat your 96 well plate with **Axol ReadySet** at a concentration of **470 µL 1X solution per cm²**. **In 96 well format, this equates to 150 µl/well**.
3. Incubate at **37°C** for **45 minutes**.
4. Wash the plate 4 times using an equal volume of ddH₂O (e.g. if 250 µL **Axol ReadySet**, use 250 µL ddH₂O per wash). Work quickly to ensure the Axol ReadySet does not dry out.

Warning: Axol ReadySet must not be allowed to dry out following the wash step. Proceed straight to coating with Axol Sure Bond

5. Dilute the **Axol Sure Bond** stock solution (**50X**) in D-PBS (without calcium or magnesium) to make **1X working solution e.g. 120 µL in 6 mL**.
2. Coat the surface of your 96 well plate with the **Axol Sure Bond 1X working solution**. We recommend coating at a concentration of **470 µL 1X solution per cm²**. **In 96 well format, this equate to 150µl/well**.
6. Incubate for **1 hour** at **37°C**.

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

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 Sure Bond** coating solution from the pre-coated culture vessel before plating resuspended cells. **It is recommended you leave behind 30% (45 µl) of the Axol Sure Bond to ensure the wells do not dry.**
7. Plate the resuspended on your **Axol Sure Bond** coated 96 well plate at 120µl per well. In total, the wells will contain 165 µl.
8. Incubate the plated cells at **37°C, 5% CO₂ 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 μ l spent medium with 100 μ l of fresh, pre-warmed **Axol Neural Maintenance-XF Medium** per well.
2. Re-feed the culture with half the volume of spent medium (75 μ l) with fresh, pre-warmed **Axol Neural Maintenance-XF Medium** (75 μ l) 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 μ l spent medium with 100 μ l 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 μ l) with fresh, pre-warmed **Axol Neural Maintenance-XF Medium** (75 μ l).
3. **24 hours after last media change**, replace 75 μ l spent medium with 75 μ l of fresh, pre-warmed **Axol Neural Maintenance-XF Medium** per well.
4. Re-feed the culture with half the volume of spent medium (75 μ l) with fresh, pre-warmed **Axol Neural Maintenance-XF Medium** (75 μ l) 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.

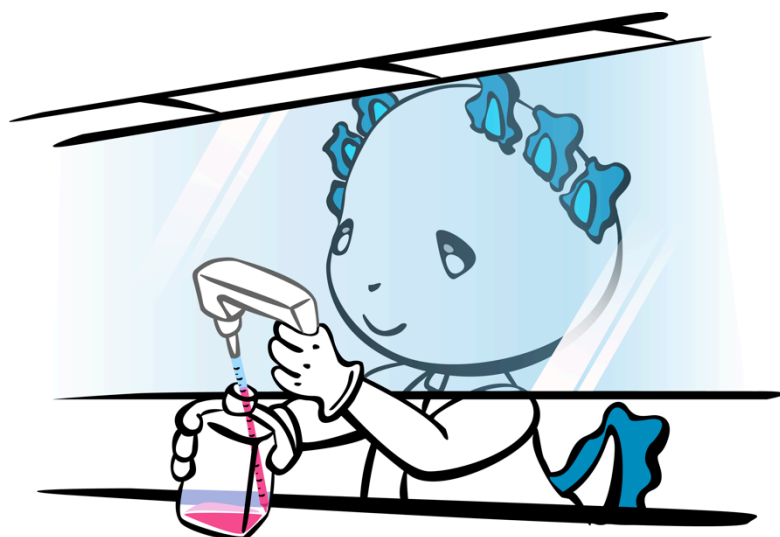
- Contact Us

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.



Don't forget to rate, review and register your Axol product at
www.axolbio.com