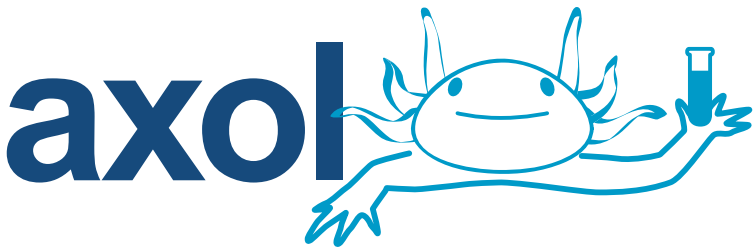
Maintenance of Axol Human Neural Stem Cells on Glass Surface (MEA Format)

Instruction Manual

Version 2.5

XF Protocol - 7



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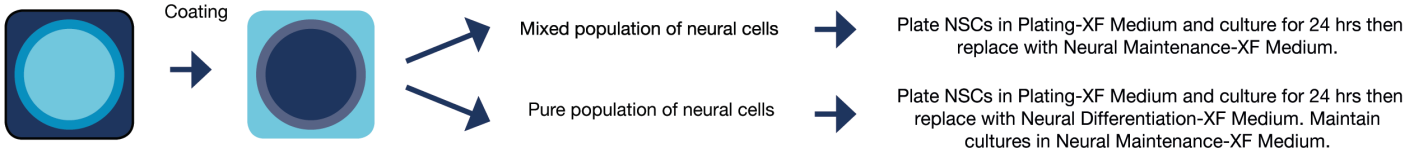
Product Information

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
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 4ºC 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 4ºC	Must be used immediately once thawed

System to culture NSCs in Glass Surface (MEA format)



Glass surface applications

Plate NSCs in Plating-XF Medium and culture for 24 hrs then replace with Neural Maintenance-XF Medium.

Plate NSCs in Plating-XF Medium and culture for 24 hrs then replace with Neural Differentiation-XF Medium. Maintain cultures in Neural Maintenance-XF Medium.

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 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/cm^2. Please check the cell count provided on the COA.

2. Thaw the **Axol Sure Bond** coating solution **overnight at 4˚C**.

3. Pre-coat your MEA with 1X **Axol ReadySet** by adding 10µl drop over the MEA electrode area.

4. Incubate at **37ºC** for **45 minutes**.

5. Wash the plate 4 times using 200µl de-ionized water. Air dry the MEA plate in a biological safety cabinet for 1 hour.

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

6. 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**.

7. Add a 10µl drop of **Axol Sure Bond** working solution over the MEA electrode area.

8. Incubate for **1 hour** at **37ºC.** Do not allow the **Axol Sure Bond** 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 a volume of **Axol Plating-XF Medium** supplemented that will give rise to 100,000 cells/10 µl.

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

7. Add a 10 µl drop (100,000 cells) over the MEA electrode area.

8. Incubate the cells at **37°C, 5% CO_2 for 1 hour.**

9. Remove the MEA after 1 hour and carefully add 300 µl of **Axol Plating-XF Medium.**

Top Tip: Synchronous differentiation at this stage will give rise to pure neurons in less than 5 days.

Spontaneous differentiation as this stage will give rise to neurons, astrocytes and oligodendrocytes after 60 days in culture.

Spontaneous Differentiation of Axol NSCs

1. **24 hours** after plating, replace the spent medium with 300 µl of fresh, pre-warmed **Axol Neural Maintenance-XF Medium** per well. Re-feed the culture with half the volume of spent medium with fresh, pre-warmed **Axol Neural Maintenance-XF Medium** every four days.

OR

Synchronous Differentiation of Axol NSCs

1. **24 hours** after plating, replace the spent medium with 300 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 with fresh, pre-warmed **Axol Neural Maintenance-XF**.

3. **24 hours** after last media change, re-feed the culture with half the volume of spent medium with fresh, pre-warmed **Axol Neural Maintenance-XF Medium**.

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

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.

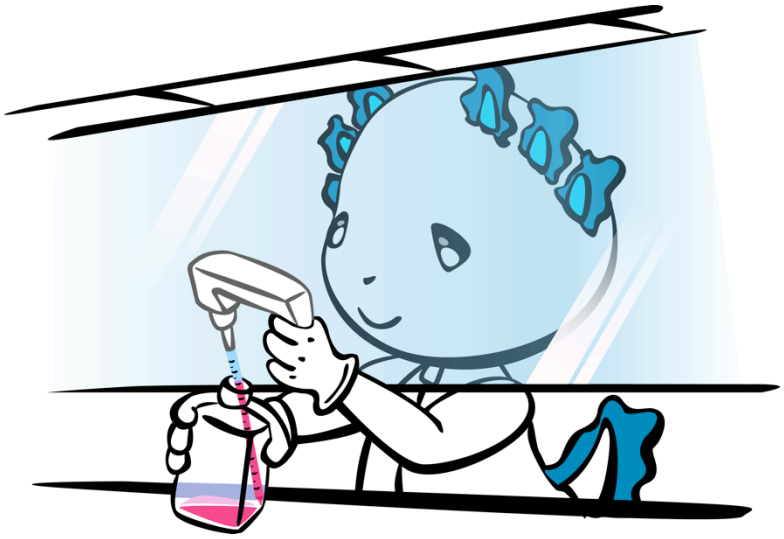
- ## Contact Us

  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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