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

<table>
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<th>Catalog no.</th>
<th>Product Name:</th>
<th>Format</th>
<th>Stock Concentration.</th>
<th>Storage on Arrival:</th>
<th>Thawing Instructions:</th>
<th>Storage Once Thawed:</th>
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<tbody>
<tr>
<td>ax0034-125</td>
<td>Axol Neural Differentiation-XF Medium</td>
<td>1 x 125 mL</td>
<td>1X</td>
<td>Aliquot and store at -80°C for up to 6 months. Keep in dark</td>
<td>Overnight at 4°C</td>
<td>Once, thawed, store aliquot at 4-8°C for up to 1 week</td>
</tr>
<tr>
<td>ax0032-500</td>
<td>Axol Neural Maintenance-XF Medium</td>
<td>1 x 500 mL</td>
<td>NA</td>
<td>Aliquot and store at -80°C for up to 6 months. Keep in dark</td>
<td>Overnight at 4°C</td>
<td>Once, thawed, store aliquot at 4-8°C for up to 1 week</td>
</tr>
<tr>
<td>ax0041+</td>
<td>Axol Sure Bond+ (Includes Axol Sure Bond™ / Axol ReadySet Solution)</td>
<td>3 x 120 µL 1 x 10 mL</td>
<td>50X 1X</td>
<td>-80°C RT</td>
<td>Overnight at 4°C NA</td>
<td>Store at 4-8°C for up to 2 weeks Store at 4-8°C for up to 1 month</td>
</tr>
<tr>
<td>ax0033</td>
<td>Axol Plating-XF Medium</td>
<td>1 x 30 mL</td>
<td>1X</td>
<td>-20°C</td>
<td>Overnight at 4°C</td>
<td>Must be used immediately once thawed</td>
</tr>
</tbody>
</table>
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/cm². Please check the cell count provided on the hyCCNs 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 37°C 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 37°C. 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 \(37^\circ\)C water bath.

2. Quickly thaw the vial of cells by swirling it in the \(37^\circ\)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 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 \(37^\circ\)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.
• 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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