axol

Human iPSC-Derived Sensory Neuron Progenitors

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Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0055	Human iPSC-Derived Sensory Neuron Progenitors	500,000 cells/ vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax0060	Sensory Neuron Maintenance Medium	250 mL	1x	Aliquot & store at -80ºC for up to 6 months	Overnight at 4ºC	Once thawed store aliquot at 4ºC for up to 1 week
ax0033	Neural Plating–XF Medium	30 mL	1x	-80ºC	Overnight at 4ºC	Must be used immediately once thawed
ax0053	SureBond-XF	1 mL	200x	4ºC	N/A	Store at 4ºC for up to 1 month
ax0052	SureBond + ReadySet	SureBond 3 x 120 µL ReadySet 2 x 10 mL	SureBond 50x ReadySet 1x	SureBond -80ºC ReadySet 4ºC	SureBond Overnight at 4ºC ReadySet N/A	SureBond Store at 4ºC for up to 2 weeks ReadySet Store at 4ºC for up to 1 month

Additional Reagents		
Product Name	**Provider**	**Catalog. No.**
Mitomycin C	Sigma	M4287
Glial-Derived Neurotrophic Factor (GDNF)	Peprotech	450-10
Nerve Growth Factor (NGF)	Peprotech	450-01
Brain-Derived Neurotrophic Factor (BDNF)	Peprotech	450-02
Neurotrophin-3 (NT-3)	Peprotech	450-03

Important!
Axol Neural Cell Culture Media

DOES NOT contain antibiotics or antifungal agents. Axol Bioscience does not recommend the use of antimicrobial agents such as penicillin, streptomycin and amphotericin. Antimicrobial agents should not be necessary if proper aseptic technique is adopted.

Preparation of Reagents

Neural Plating-XF Medium

- Upon receipt, store **Neural Plating–XF Medium** at or below **-80°C** protected from light.
- When ready to use, thaw **Neural Plating–XF Medium** overnight at **4°C** in the dark.
- Once thawed, **Neural Plating–XF Medium** must be used and cannot be refrozen.

Sensory Neuron Maintenance Medium

- Upon receipt, aliquot and store **Sensory Neuron Maintenance Medium** at or below **-80°C** protected from light.
- When ready to use, thaw an aliquot of **Sensory Neuron Maintenance Medium** overnight at **4°C** in the dark.
- Prepare **Sensory Neuron Maintenance Medium** by adding the following growth factors:

Growth Factor	Final Concentration
Glial-Derived Neurotrophic Factor (GDNF)	25 ng/mL
Nerve Growth Factor (NGF)	25 ng/mL
Brain-Derived Neurotrophic Factor (BDNF)	10 ng/mL
Neurotrophin-3 (NT-3)	10 ng/mL

- Growth factors should be added fresh each time an aliquot of **Sensory Neuron Maintenance Medium** is thawed.
- A thawed and supplemented aliquot of **Sensory Neuron Maintenance Medium** can be stored at **4°C** for **1 week**.

Mitomycin C

- Prepare a **0.5 mg/mL** stock concentration of **mitomycin C** by solubilizing **2 mg in 4 mL** of ddH_2O. Make 50-100 µL aliquots of **mitomycin C** (0.5 mg/mL), protect from light and store in a dark box at **4°C**. Stored at **4°C**, **mitomycin C** is stable for up to **8 weeks**.

Sensory Neuron Maintenance Medium containing Mitomycin C

- Prepare medium containing **2.5 µg/mL** of **mitomycin C** by adding **100 µL** of the 0.5 mg/mL stock of **mitomycin C** to **20 mL** of **Sensory Neuron Maintenance Medium**.
- This medium should then be filter sterilized prior to use using a **0.22 µM filter**.

SureBond-XF Coating Solution (required for plating on plastic)

- Upon receipt, store **SureBond-XF** at **4°C**.
- Calculate the total surface area that requires coating.
- Dilute the **SureBond-XF** stock solution (200x) in Dulbecco's-PBS (1x) (D-PBS without calcium or magnesium) to make 1x working solution e.g. **30 µL** in **6 mL**.
- Coat the surface of your culture vessel with the **SureBond-XF** 1x working solution. We recommend coating at a volume of **200 µL per cm²** however, please optimize for your experiments.

SureBond+ReadySet Coating Solution (required for plating on glass)

- Upon receipt, store **SureBond** at or below **-80°C** and store **ReadySet** at **4°C**.
- Thaw the **SureBond** coating solution overnight at **4°C**.
- Calculate the total surface area that requires coating.
- Pre-coat your culture vessel with **ReadySet** at a volume of **250 µL per cm²**.
- Incubate at **37ºC** for **45 minutes**.
- Wash the plate thoroughly **four times** using an **equal volume** of sterile ddH_2O (e.g. if 250 µL of **ReadySet**, use 250 µL sterile ddH_2O). During each wash, rock the dish to ensure thorough washing.
- Do not let the **ReadySet** dry out following washing, proceed straight to coating with **SureBond**.
- Dilute the **SureBond** stock solution (50x) in D-PBS (1x) (without calcium or magnesium) to make 1x working solution e.g. **120 µL** in **6 mL**.
- Coat the surface of your culture vessel with the **SureBond** 1x working solution. We recommend coating at a volume of **200 µL per cm²**.
- Incubate for **1 hour** at **37°C**.

Important!

Make sure that the coating does not evaporate.
Do not let the **SureBond** or **SureBond-XF** coating dry out before seeding the cells.
DO NOT wash the vessel after coating with **SureBond** or **SureBond-XF**.

Culture of Human iPSC-Derived Sensory Neuron Progenitors

Thawing and Plating

- Thaw **Neural Plating-XF Medium** overnight at **4°C**.
- Prepare culture vessels by coating with either **SureBond-XF** **4 hours** prior to thawing cells or **SureBond+ReadySet**.
- Pre-warm all media and culture vessels to **37°C** before use.
- To thaw the cells – transfer the vial of cells from storage by transporting the vial buried in dry ice. Remove the vial from dry ice and transfer it to a **37°C** water bath.
- Quickly thaw the vial of cells in a **37°C** water bath. Do not completely submerge the vial (only up to 2/3rd of the vial). Remove the vial before the last bit of ice has melted, after 1-2 minutes.
- **Do not shake the vial during thawing**.
- Take the vial of cells to a biological safety cabinet, spraying the vial and hood thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing the vial in the hood.
- Using a P1000 pipette, transfer the cell suspension into a 15 mL sterile conical tube. Gently wash the cryogenic vial with **1 mL** of warm **Neural Plating-XF Medium** and transfer this to the 15 mL sterile conical tube.
- Add **8 mL** of **Neural Plating-XF Medium** drop-wise to the cell suspension.
- Centrifuge cells at **200 x *g*** for **5 minutes** at **room temperature**.
- Aspirate and discard the supernatant carefully with a pipette.
- Using a P1000 pipette, gently resuspend the cell pellet in **1 mL** of **Neural Plating-XF Medium** until they are in a single cell suspension.
- Perform a cell count to ensure optimal seeding density.
- Plate the resuspended cells drop-wise and evenly at a density ranging from **100,000 - 150,000 cells/cm²**.
- Gently rock the culture vessel back and forth to ensure an even seeding density.
- Incubate the cells at **37°C**, **5% CO_2**.
- The day after plating, replace the medium with fresh pre-warmed, **37°C**, **Sensory Neuron Maintenance Medium supplemented with GDNF (25 ng/mL)**, **NGF (25 ng/mL)**, **BDNF (10 ng/mL)**, **NT-3 (10 ng/mL)**.

Growth Arrest and Purification

- **Two days** after thawing the **Human iPSC-Derived Sensory Neuron Progenitors** remove all of the culture medium and replace with **Sensory Neuron Maintenance Medium** containing **2.5 µg/mL** of **mitomycin C**.
- Incubate the cells for **2 hours** at **37ºC**, **5% CO_2**.
- After the incubation period, remove the **Sensory Neuron Maintenance Medium** containing **2.5 µg/mL** of **mitomycin C** from the culture and gently wash the cells once with D-PBS (1x) (without calcium or magnesium).
- After washing, add pre-warmed, **37ºC**, **Sensory Neuron Maintenance Medium supplemented with GDNF (25 ng/mL)**, **NGF (25 ng/mL)**, **BDNF (10 ng/mL)**, **NT-3 (10 ng/mL)**. The effect of **mitomycin C** treatment is not immediate. Non-neuronal cell death will not occur until **4-5 days** after treatment. Full effects will be apparent after **7 days**.

Maintenance of Human iPSC-Derived Sensory Neuron Progenitors

- To maintain a healthy culture, replace half the volume of medium with fresh pre-warmed, **37ºC**, **Sensory Neuron Maintenance Medium supplemented with GDNF (25 ng/mL)**, **NGF (25 ng/mL)**, **BDNF (10 ng/mL)**, **NT-3 (10 ng/mL)** **every 3-4 days**.
- Maintain the neurons in **Sensory Neuron Maintenance Medium supplemented with GDNF (25 ng/mL)**, **NGF (25 ng/mL)**, **BDNF (10 ng/mL)**, **NT-3 (10 ng/mL)** for a minimum of **6 weeks** prior to performing endpoint assays.
- **After 4-6 weeks** of maturation, the sodium channel $Na_v1.7$ should be expressed and **after 6-8 weeks** of maturation, $Na_v1.8$ should be expressed by the sensory neurons.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
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