

# Human iPSC-derived Sensory Neuron Progenitors

For generation of functional human sensory  
neurons

User Guide  
Version 6.01

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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	≥0.5 million cells/vial	N/A	Vapor Phase Nitrogen	Follow protocol	N/A
ax0033	Neural Plating medium	30 mL	1 X	-80°C	Overnight at 4°C	Must be used immediately once thawed
ax0060	Sensory Neuron Maintenance Medium	250 mL	1 X	Aliquot & store at -80°C for up to 6 months	Overnight at 4°C	Once thawed store aliquot at 4°C for up to 1 week
ax0058	Sensory Maturation Maximizer Supplement	1 mL	100 X	Aliquot & store at -80°C (See Vial for date of expiry)	Thaw at room temperature in biosafety cabinet	Must be used immediately once thawed
ax0053	SureBond-XF	1 mL	200 X	4°C	N/A	Stable at 4°C for up to 6 months
ax139855 (10 µg)	Recombinant Human Glial-Derived Neurotrophic Factor (GDNF)	10 µg Lyophilized Powder	N/A	-20°C	N/A	Reconstituted protein should be used immediately or stored in working aliquots at -80°C
ax139800 (10 µg)	Recombinant Human Brain-Derived Neurotrophic Factor (BDNF)	10 µg Lyophilized Powder	N/A	-20°C	N/A	
ax139789 (20 µg)	Recombinant Human Nerve Growth Factor (NGF)	20 µg Lyophilized Powder	N/A	-20°C	N/A	
ax139811 (10 µg)	Recombinant Human Neurotrophin-3 (NT-3)	10 µg Lyophilized Powder	N/A	-20°C	N/A	

Additional Reagents		
Product Name	Supplier	Product Code
Mitomycin C	Sigma-Aldrich	M4287
Poly-D-Lysine	Sigma-Aldrich	P7405
Y-27632 2HCl (ROCK inhibitor)	Focus Biomolecules	10-2301

**Important! Axol Neural Cell Culture Media**

## Human iPSC-derived Sensory Neuron Progenitors

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

## Preparation of Media and Coating Reagents

### Neural Plating Medium

- Upon receipt, store Neural Plating Medium at -80°C.
- When ready to use, thaw Neural Plating Medium overnight at 4°C.
- Once thawed, Neural Plating Medium must be used and cannot be refrozen.
- **Neural Plating Medium** requires supplementing with **Y-27632 dihydrochloride (ROCK Inhibitor)** to a final concentration of 10µM before use.

### Supplement Sensory Neuron Maintenance Medium with Growth Factors and Sensory Maturation Maximizer

- Upon receipt, aliquot and store Sensory Neuron Maintenance Medium at -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 10 µg/mL stock solutions of each growth factor by resuspending the lyophilized powder in D-PBS (without calcium or magnesium) supplemented with 0.05% human or bovine serum albumin (HSA or BSA). The growth factors can be aliquoted and stored at -80°C.
- Thaw an aliquot of the Sensory Maturation Maximizer Supplement (Maximizer) at room temperature.
- Prepare Sensory Neuron Maintenance Medium by adding the following:

Growth factor	Stock concentration	Final concentration	Volume to add in 20 mL
Sensory Maturation Maximizer Supplement	100 X	1 X	200 µL
Recombinant Human Glial-Derived Neurotrophic Factor (GDNF) (ax139855)	10 µg/mL	25 ng/mL	50 µL
Recombinant Human Nerve Growth Factor (NGF) (ax139789)	10 µg/mL	25 ng/mL	50 µL
Recombinant Human Brain-Derived	10 µg/mL	10 ng/mL	20 µL



Neurotrophic Factor (BDNF) (ax139800)			
Recombinant Human Neurotrophin-3 (NT-3) (ax139811)	10 µg/mL	10 ng/mL	20 µL

- The growth factors and the Maximizer should be added fresh each time an aliquot of Sensory Neuron Maintenance Medium is thawed. Do not refreeze supplemented medium.

### Mitomycin C

- Prepare a 0.5 mg/mL stock concentration of mitomycin C by solubilizing 2 mg in 4 mL of DMSO. Make 50~100 µL aliquots of stock mitomycin C solution, protect from light and store at -80°C. Diluted mitomycin C is stable for up to 4 weeks at -80°C.

### Supplement Sensory Neuron Maintenance Medium with Mitomycin C

- Prepare medium containing 2.5 µg/mL of mitomycin C by adding 100 µL of the 0.5 mg/mL mitomycin C stock to 20 mL of growth factor and Maximizer supplemented Sensory Neuron Maintenance Medium.
- This medium should then be filter sterilized with a 0.22 µM filter prior to use.
- Prepare and use on the day required, do not store.

### SureBond-XF Coating Solution

This coating is used for differentiating the sensory neuron progenitors on **plastic surface**.

- Dilute the SureBond-XF stock solution (200X) in D-PBS (without calcium or magnesium) to make 1X working solution, e.g. 30 µL in 6 mL.
- Coat the culture surface with 200 µL per cm<sup>2</sup> of the SureBond-XF 1X working solution.
- Incubate for at least 4 hours at 37°C.
- Remove the SureBond-XF from the culture vessel prior to plating of cells. Do not wash the culture vessel after coating with SureBond-XF.
- Do not let the SureBond-XF coating dry out before plating the cells.

### **Poly-D-Lysine + SureBond-XF Coating Solution**

This set of coating solutions is used for differentiating the sensory neuron progenitors on **glass coverslips or multi-electrode array plate**.

- Add 50 mL of sterile tissue culture grade water to 5 mg of Poly-D-Lysine (Sigma-Aldrich, P7405), and aseptically coat the surface with 40  $\mu\text{L}$  per  $\text{cm}^2$  (equivalent to 1 mL per 25  $\text{cm}^2$ ) of solution. After 5 minutes, remove the solution through aspiration and thoroughly rinse the surface twice with sterile water. Let dry for two hours before proceeding onto SureBond-XF coating.

## **Differentiation of iPSC-derived Sensory Neuron Progenitors**

### **Thawing and Plating**

- Prepare coating of culture vessels as described above.
- Thaw Neural Plating Medium overnight at 4°C.
- Pre-warm Neural Plating Medium and culture vessels to 37°C before use.
- To thaw the cells, transfer the vial of cells from nitrogen storage with the vial buried in dry ice.
- Remove the vial from dry ice and transfer it immediately to 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.
- Do not shake the vial during thawing.
- Spray the vial with 70% ethanol and wipe it down with a sterile paper towel before placing it in the cell culture hood.
- Once thawed completely, use a P1000 pipette to transfer the cells into a 15 mL sterile conical tube.
- Gently wash the cryovial with 1 mL of warm Neural Plating Medium and transfer this to the 15 mL sterile conical tube.
- Add 8 mL of Neural Plating medium dropwise to the cell suspension in the conical tube. Gently mix the cells with the medium with a 10 mL serological pipette.

**Important!** Do not mix the cells vigorously. Avoid generating bubbles while pipetting.

- Centrifuge cells at **200 x g** for **5 minutes** at room temperature.
- Aspirate the medium carefully and gently resuspend the cell pellet in **2 mL** of the plating medium until they become mostly a single cell suspension.
- Perform a cell count. We recommend seeding the sensory neuron progenitors at 100,000 cells/cm<sup>2</sup>.
- Remove coating solution from the culture vessel. Add an appropriate volume of the plating medium to the culture vessel. Do not let the coating to dry out during the process.
- Plate the resuspended cells dropwise and evenly.
- Gently rock the culture vessel back and forth to ensure an even seeding density.
- Incubate the cells at 37°C, 5% CO<sub>2</sub>.
- The day after plating (Day 1), replace the medium with fresh pre-warmed Sensory Neuron Maintenance Medium supplemented with the growth factors and the Maximizer.

### Growth Arrest and Purification

- Two days after the medium change (Day 3), replace the spent medium with mitomycin C supplemented Sensory Neuron Maintenance Medium (prepared as described above).
- Incubate the cells for **2 hours** at 37°C, 5% CO<sub>2</sub>.
- After the incubation period, remove all the medium from the culture vessel and gently rinse the cells once with D-PBS (without calcium or magnesium).
- Add pre-warmed, Sensory Neuron Maintenance Medium supplemented with the growth factors and the Maximizer.

**Please note:** The effect of mitomycin C treatment is not immediate. Non-neuronal cell elimination **will not occur until 4 days** post-treatment. The full effect will be apparent after **7 days**.



## Maturation of iPSC-derived Sensory Neurons

- On **Day 5**, perform a **full** medium change with fresh, pre-warmed Sensory Neuron Maintenance Medium supplemented with the growth factors and the Maximizer.
- To maintain a healthy culture, replace **half** the volume of the medium with fresh pre-warmed, supplemented Sensory Neuron Maintenance Medium **every 3 days at Day 8, 11, 14,17, 20, 23 and 26**.

After **21 days** of culturing, **TrpV1** and **Nav1.7 and Nav1.8 sodium channels** should be expressed by the sensory neurons, and the cells are assay-ready. We suggest that you carry out your experiments with the cells **between day 21 and day 29**.

## Outline of the Cell Culture Preparation

Day	Instruction	Medium	Note
-1	Coat culture vessel with substrates; Thaw plating medium overnight		
0	Thaw and plate the cells	Neural Plating Medium	
1	Full medium change to Sensory Neuron Maintenance Medium supplemented with the growth factors and the Maximizer	Sensory Neuron Maintenance Medium <b>supplemented with the growth factors and the Maximizer</b>	
3	Two-hour treatment with mitomycin C	Sensory Neuron Maintenance Medium <b>supplemented with the growth factors and the Maximizer</b> with and then without mitomycin C	
5	Full medium change	Sensory Neuron Maintenance Medium <b>supplemented with the growth factors and the Maximizer</b>	
Day 8, 11, 14,17, 20, 23 and 26	Half medium change	Sensory Neuron Maintenance Medium <b>supplemented with the growth factors and the Maximizer</b>	Assay time window: Day 21 ~ 29

For **each vial of cells (500,000 cells per vial)**, approximately **10 mL** of supplemented Sensory Neuron Maintenance Medium will be required for the 29 days of culture.

Got any questions? Need help with the User Guide?  
Contact Axol Technical Support at  
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