AXOL

Discovery Stems From Here

Human iPSC-Derived Sensory Neuron Progenitors

For the generation of iPSC-derived sensory neurons

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	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 4°C	Once thawed store aliquot at 4ºC for up to 1 week
ax0033	Neural Plating-XF medium	30 mL	1x	-80ºC	Overnight at 4°C	Must be used immediately once thawed
ax0053	SureBond-XF	1 mL	200x	4°C	N/A	Stable at 4°C for up to 6 months
ax0052	SureBond+ReadySet	SureBond 3 x 120 µL ReadySet 2 x 10 mL	SureBond 1 mg/mL ReadySet 1x	SureBond -80°C ReadySet 4°C	SureBond Overnight at 4°C ReadySet N/A	SureBond Store at 4°C for up to 2 weeks ReadySet Store at 4°C for up to 1 month
ax139855 (10 µg)	Recombinant Human Glial-Derived Neurotrophic Factor (GDNF)	10 µg Lyophilized Powder	N/A	-20ºC	N/A	Reconstituted protein should be used immediately or stored in working aliquots at -20°C
ax139800 (10 µg)	Recombinant Human Brain-Derived Neurotrophic Factor (BDNF)	10 µg Lyophilized Powder	N/A	-20ºC	N/A	
ax139789 (20 µg)	Recombinant Human Nerve Growth Factor (NGF)	20 µg Lyophilized Powder	N/A	-20ºC	N/A	
ax139811 (10 µg)	Recombinant Human Neurotrophin-3 (NT-3)	10 µg Lyophilized Powder	N/A	-20ºC	N/A	
ax0057	Human iPSC-Derived Sensory Neuron Progenitor Kit (male)	Kit components: ax0055, ax0060 ax0033, ax0052 ax0053, ax139855 ax139800, ax139789 ax139811	See above instructions	See above instructions	See above instructions	See above instructions

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Mitomycin C	Sigma-Aldrich	M4287

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 10 μg/mL stock solutions of each growth factor by resuspending the lyophilized powder in PBS (1x) supplemented with 0.05% human serum albumin (HSA).
- The growth factors can be aliquoted and stored at 4°C for up to 1 week or -20°C for longer storage.
- Prepare Sensory Neuron Maintenance Medium by adding the following growth factors:

Growth factor	Stock concentration	Final concentration	Volume to add in 20 mL
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 Neurotrophic Factor (BDNF) (ax139800)	10 μg/mL	10 ng/mL	20 μL
Recombinant Human Neurotrophin-3 (NT-3) (ax139811)	10 μg/mL	10 ng/mL	20 μL

- 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 ddH2O. 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

This coating solution is primarily used for culture of iPSC-derived sensory neuron progenitors on a plastic culture vessel.

- 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.
- Incubate for 4 hours at 37°C.
- Remove the SureBond-XF from the culture dish prior to seeding. Do not wash the culture vessel after coating with SureBond-XF.
- Do not let the SureBond-XF coating dry out before seeding the cells.

SureBond+ReadySet Coating Solution

This coating solution is primarily used for culture of iPSC-derived sensory neuron progenitors on a glass culture vessel or multi-electrode array plate.

- 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.
- If using glass coverslips, clean coverslips thoroughly before coating with SureBond+ReadySet.

ReadySet

- Pre-coat your culture vessel with ReadySet at a volume of 250 µL per cm^2.
- Incubate at 37ºC for 45 minutes.
- Wash the plate thoroughly four times using an equal volume of sterile distilled H_2O (e.g. if 250 µL of ReadySet, use 250 µL sterile distilled H_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**.

SureBond

- Dilute the SureBond stock solution (1 mg/ml) in D-PBS (1x) (without calcium or magnesium) to a final concentration of 20 µg/mL 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 cm^2.
- Incubate overnight at 37°C.
- Remove the SureBond from the culture dish prior to seeding. Do not wash the culture vessel after coating with SureBond.
- Do not let the SureBond coating dry out before seeding the cells.

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 on the previous day.
- 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/cm^2.
- 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
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Or
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