axol

Human Dopaminergic Neuron Progenitors

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Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0091	Human iPSC-Derived Dopaminergic Neuron Progenitors	1 million cells/ vial	N/A	Liquid Nitrogen	Follow protocol	N/A
	Dopaminergic Neuron Basal Medium	50 mL	1x	-20ºC	Overnight at 4ºC	Store at -20ºC for up to 6 months
	Supplement A	1 vial 80 µL	1x	-20ºC	Overnight at 4ºC	Store at -20ºC for up to 6 months
	Supplement B	1 vial 60 µL	1x	-20ºC	Overnight at 4ºC	Store at -20ºC for up to 6 months
ax0041+	SureBond	3 x 120 µL	50x	-80ºC	Overnight at 4ºC	Store at 4ºC for up to 2 weeks
	ReadySet	2 x 10 mL	1x	4ºC	N/A	Store at 4ºC for up to 1 month

Lot-specific information such as specifications and quality control details are stated in the Certificate of Analysis.

Recommendations

- Recommended culture vessel coating: **SureBond+ReadySet**
- Recommended cell culture medium: **Differentiation Medium** for **5 days** followed by **Maintenance Medium**
- Recommended seeding density for assay: **40,000-60,000 viable cells/cm^2**
- Recommended centrifugation speed: **400 x *g*** for **5 minutes**
- Recommended days in culture before assay: **14 days** (minimum for tyrosine hydroxylase expression) (**5 days** in **Differentiation Medium** then **9 days** in **Maintenance Medium**)

Preparing Dopaminergic Neuron Media

Dopaminergic Neuron Differentiation Medium

- Upon receipt aliquot and store the **Dopaminergic Neuron Basal Medium** at or below **-20°C** protected from light. Make **1 x 20 mL** aliquot and **2 x 15 mL** aliquots. Stored at **-20°C**, the medium is stable for 6 months from date of manufacture.
- When ready to use, thaw the **20 mL** aliquot of **Basal Medium** overnight at **4°C** in the dark.
- On the day of thawing **Human iPSC-Derived Dopaminergic Neuron Progenitors**, transfer **20 mL** of aliquoted **Basal Medium** to a fresh 50 mL tube.
- Pre-thaw **Supplement A** on ice and add the entire contents (**80 µL**) to the **20 mL** aliquot of **Basal Medium**. This will make **Differentiation Medium**.

Dopaminergic Neuron Maintenance Medium

- When ready to use, thaw a **15 mL** aliquot of **Basal Medium** overnight at **4°C** in the dark.
- On day 5 post-seeding, transfer **15 mL** of aliquoted **Basal Medium** to a fresh 50 mL tube.
- Thaw **Supplement B** on ice and add **30 µL** to the **15 mL** aliquot of **Basal Medium**. This will make **Maintenance Medium**.
- Refreeze the remaining **Supplement B** (**30 µL**) until more **Maintenance Medium** is required.

Important!

Axol recommends: SureBond+ReadySet coating reagent for **Human iPSC-Derived Dopaminergic Neuron Progenitors**.

Coating the Culture Vessel with SureBond+ReadySet

- Calculate the total surface area that requires coating.
- Thaw the **SureBond** coating solution **overnight** at **4°C**.
- Pre-coat the culture vessel with **ReadySet** at a concentration of **250 µL per cm²**.
- 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 uL of **ReadySet** is used 250 uL 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**.
- Dilute the **SureBond** stock solution (50x) in Dulbecco's-PBS (1x) (D-PBS, without calcium or magnesium) to make 1x working solution e.g. **120 µL** in **6 mL**.
- Coat the surface of the culture vessel with the **SureBond** 1x working solution. Axol recommends coating at a volume of **200 µL per cm²**, however, please optimize for your experiments.
- Incubate for **1 hour** at **37ºC**.

Thawing and Plating Human Dopaminergic Neuron Progenitors

- On the day before thawing **Human Dopaminergic Neuron Progenitors** cells, prepare the **Differentiation Medium**.
- Pre-warm all media and vessels to **37ºC** before use.
- Aliquot **5 mL** of **Differentiation Medium** into a 15 mL sterile tube and pre-warm at **37ºC**.
- Prepare a second tube with enough **Differentiation Medium** for cell culture vessels (see **Table 1**) and pre-warm at **37ºC**. Store the remaining medium at **4ºC**.
- To thaw cells – transfer the vial of cells from liquid nitrogen storage with the vial buried in dry ice. Remove the vial from dry ice and transfer it immediately to a **37ºC** water bath.
- Quickly thaw the vial of cells by swirling it in a **37ºC** water bath. Do not completely submerge the vial (only up to two thirds 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 it thoroughly with 70% ethanol and wiping with an autoclaved paper towel before placing it in the hood.
- Once thawed, use a P1000 pipette to transfer the cells drop-wise into a 15 mL sterile conical tube containing **5 mL** of pre-warmed (**37ºC**) **Differentiation Medium**. Gently wash the vial with **1 mL** of **Differentiation Medium**. Transfer this to the 15 mL sterile conical tube containing the cells.

Important!

Do not mix the cells vigorously. Avoid generating bubbles.

- Centrifuge cells at **400 x *g*** for **5 minutes** at room temperature
- Aspirate the supernatant carefully and resuspend the cell pellet with **1 mL** of **Differentiation Medium**.
- Gently resuspend the cells until they are in a single cell suspension.
- Remove **10 µL** of cell suspension and mix it with **10 µL** of trypan blue solution. Count the cells.
- Calculate the appropriate volume of **Differentiation Medium** with respect to the culture vessel (see **Table 1**).
- Resuspend the cells in **Differentiation Medium** and plate the resuspended cells at a **density ranging from 40,000 - 60,000 cells/cm²** on the **SureBond+ReadySet** coated culture vessel.
- Plate the cells drop-wise and evenly on the culture vessels. Incubate the cells at **37ºC**, **5% CO_2**.
- After three days, replace the medium with fresh, pre-warmed, **37ºC**, **Differentiation Medium**. Medium should be changed gently in a drop-wise fashion while pointing the pipette tip toward the wall of the culture vessel. Refresh the medium **every 2 days**.

Table 1: Recommended volumes of medium for different culture vessels

Vessel Type	Medium Volume
96-well plate	100 µL/well
24-well plate	500 µL/well
35 mm dish	2 mL
60 mm dish	5 mL

Maintenance and Maturation of Human Dopaminergic Neuron Progenitors

- On day 5 post-seeding, prepare the **Maintenance Medium** and pre-warm (**37ºC**) an aliquot. Store the remaining medium at **4ºC**.
- Gently replace the **Differentiation Medium** with **Maintenance Medium**.
- Continue maturation of dopaminergic neurons in **Maintenance Medium**. Change the medium **every 2 days**. Dopaminergic neurons can be stained and analysed 14 days after seeding.
- After 14 days in culture, 30% of the dopaminergic neurons should be tyrosine hydroxylase positive.

Top Tip

Dopaminergic neurons should be matured for up to 5 weeks after seeding in order to identify dopaminergic neuron receptors and to obtain electrically active dopaminergic neurons. Longer culture will also increase population of astrocytes (GFAP positive).

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