AXOL

Discovery Stems From Here

Human iPSC-Derived Motor Neuron Progenitors

For the generation of iPSC-derived Motor Neurons

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0078	Human iPSC-Derived Motor Neuron Progenitors (Healthy)	≥2 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax0073	Human iPSC-Derived Motor Neuron Progenitors (Healthy - sibling (C9ORF72 extension))	≥2 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax0074	Human iPSC-Derived Motor Neuron Progenitors (ALS (C9ORF72 extension))	≥2 million cells/vial	N/A	Liquid Nitrogen	Follow protocol	N/A
ax0071	Motor Neuron Maintenance Medium	50 mL	1x	-80°C	Overnight at 4°C	Once thawed, aliquot and store at 4ºC for up to 1 week or for long term store at -80°C.
ax0072	Motor Neuron Maintenance Medium	200 mL	1x	-80°C	Overnight at 4°C	Once thawed, aliquot and store at 4ºC for up to 1 week or for long term store at -80°C.
ax0041	SureBond	3 x 120 µL	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
ax0044	Unlock	25 mL	1x	Aliquot and store at -80°C for 6 months	Overnight at 4°C	Once thawed, store aliquot at 4ºC for up to 1 week
ax139888 (5 µg)	Recombinant Human Ciliary-Neurotrophic Factor (CNTF)	5 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 owder	N/A	-20ºC	N/A	
ax68168 (5 mg)	Y-27632 2HCl (ROCK inhibitor)	5 mg Lyophilized Powder	N/A	Stable for 2 years at -20°C. Solutions in DMSO or methanol may be stored at -20°C for up to 3 months	N/A	Reconstituted protein should be used immediately or stored in working aliquots at -20°C
ax0178	Human iPSC-Derived Motor Neuron Progenitor Kit (healthy, male)	Kit components: ax0078, ax0071, ax0072, ax0041, ax0052, ax0044, ax139888, ax139800, ax68168	See above instructions	See above instructions	See above instructions	See above instructions

Additional Reagents		
Product Name	**Supplier**	**Product Code**
Retinoic acid	Sigma-Aldrich	R2625

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

Motor Neuron Recovery Medium

- Upon receipt, aliquot and store Motor Neuron Recovery Medium at or below -80°C protected from light.
- When ready to use, thaw an aliquot of Motor Neuron Recovery Medium overnight at 4°C in the dark.
- Motor Neuron Recovery Medium requires supplementing with retinoic acid before use.
- Prepare the retinoic acid by creating a stock concentration of 1 mM in DMSO.
- Prepare Motor Neuron Recovery Medium by adding retinoic acid to a final concentration of 0.1 µM e.g. 5 µL in 50 mL.
- During thawing and passaging Motor Neuron Recovery Medium should be supplemented with Y-27632 2HCl to a final concentration of 10 µM.

Motor Neuron Maintenance Medium

- Upon receipt, aliquot and store Motor Neuron Maintenance Medium at or below -80°C protected from light.
- When ready to use, thaw an aliquot of Motor Neuron Maintenance Medium overnight at 4°C in the dark.
- Motor Neuron Maintenance Medium requires supplementing with three compounds before use.
- Prepare Motor Neuron Maintenance Medium by adding the following factors fresh each time:

Growth factor	Stock concentration	Final concentration	Volume to add in 50 mL
Recombinant Human Ciliary-Derived Neurotrophic Factor (CNTF) (ax139888)	5 µg/mL	10 ng/mL	100 µL
Recombinant Human Brain-Derived Neurotrophic Factor (BDNF) (ax139800)	10 µg/mL	5 ng/mL	25 µL
Retinoic acid	1 mM	0.5 µM	25 µL

SureBond Coating Solution

This coating solution is used for culture of iPSC-derived motor neuron progenitors on thawing and prior to differentiation and maturation.

- Upon receipt, store SureBond at -80°C or below.
- Thaw the SureBond coating solution overnight at 4°C.
- Calculate the total surface area that requires coating.
- 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.

SureBond+ReadySet Coating Solution

This coating solution is used for the culture, differentiation and maturation of iPSC-derived motor neuron progenitors to motor neurons.

- 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.

Unlock

- Upon receipt, aliquot and store Unlock at or below -80°C protected from light. Stored at -80°C, the reagent is stable for 6 months from date of manufacture.

Culture of Human iPSC-Derived Motor Neuron Progenitors

Thawing and Plating

- The day before thawing Human iPSC-Derived Motor Neuron Progenitors
- Thaw an aliquot of Motor Neuron Recovery Medium overnight at 4°C.
- Prepare culture vessels by coating with SureBond overnight prior to thawing cells.
    - Matrigel™ can also be used to coat the culture vessels. Prepare culture vessels with Matrigel™ on the day of thawing at least 1 hour before seeding the cells (prepared before seeding in accordance with manufacturer's instructions).
- T-25 flasks or 60 mm dishes are recommended for initial plating of Human iPSC-Derived Motor Neuron Progenitors after thawing.
- On the day of thawing Human iPSC-Derived Motor Neuron Progenitors
- Prepare Motor Neuron Recovery Medium by adding Y-27632 2HCl to a final concentration of 10 µM and retinoic acid to a final concentration of 0.1 µM.
- Pre-warm all media and culture vessels to 37°C before use.
- Add 4 mL of Motor Neuron Recovery Medium into a 15 mL sterile conical tube.
- 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, gently add the cell suspension drop-wise into the 15 mL sterile conical tube containing Motor Neuron Recovery Medium. Gently wash the cryogenic vial with 1 mL of warm Motor Neuron Recovery Medium and transfer this to the 15 mL sterile conical tube.
- Centrifuge cells at 200 x *g* for 5 minutes at room temperature.
- Carefully aspirate and discard the supernatant using a pipette.
- Using a P1000 pipette, gently resuspend the cell pellet in 1 mL of Motor Neuron Recovery Medium until they are in a single cell suspension.
- Perform a cell count to ensure optimal seeding density.
- Remove the coating solution and add an appropriate volume of medium to the culture vessel. Do not let the culture vessel dry out.
- 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 all of the medium with fresh pre-warmed, 37°C, Motor Neuron Recovery Medium (without Y-27632 2HCl).
- Every 2 days replace all of the medium with fresh pre-warmed, 37°C, Motor Neuron Recovery Medium (without Y-27632 2HCl). Passage the Human iPSC-Derived Motor Neuron Progenitors at day 5-7, depending on confluency.

Passaging of Human iPSC-derived Motor Neuron Progenitors

- When the culture is 70 % confluent, it is ready to undergo passaging.
- The day before passaging Human iPSC-Derived Motor Neuron Progenitors
- Thaw an aliquot of Unlock and Motor Neuron Maintenance Medium overnight at 4ºC before use and store at 4ºC.
- On the day of passaging Human iPSC-Derived Motor Neuron Progenitors
- Pre-Coat Culture Vessels: Make sure to pre-coat the surface of your culture vessel that the cells will be passaged into. For final seeding, pre-coat culture vessels with SureBond+ReadySet or MatrigelTM (prepared before seeding in accordance with manufacturer's instructions).
- Prepare Motor Neuron Maintenance Medium by adding Y-27632 2HCl to a final concentration of 10 µM and retinoic acid to a final concentration of 0.5 µM, BDNF to a final concentration of 5 ng/mL and CNTF to a final concentration of 10 ng/mL.
- Pre-warm all media and culture vessels to **37ºC** before use.
- Remove all spent medium from cell culture vessels.
- Gently rinse the surface of the cell layer once with the D-PBS (1x) (without calcium or magnesium). We recommend using 2 mL per 10 cm^2 of culture surface area. I Discard the D-PBS.
- To detach the cells from a coating of SureBond use Unlock.
- Add 1 mL per 10cm^2 of culture surface area of cold/room temperature Unlock. Evenly distribute it over the entire cell layer. Incubate the cells for 5 minutes at 37°C.
- Use a P1000 pipette to transfer the cells drop-wise into a 15 mL sterile conical tube and then gently add four volumes of pre-warmed, 37°C, Motor Neuron Maintenance Medium. (For example if 1 mL of Unlock is used, then add 4 mL of the medium to stop the reaction). Gently pipette up and down a few times to disperse the medium.
- Centrifuge cells at 200 x *g* for 5 minutes at room temperature.
- Carefully aspirate and discard the supernatant using a pipette.
- Using a P1000 pipette, gently resuspend the cell pellet in 1 mL of Motor Neuron Maintenance Medium until they are in a single cell suspension.
- Perform a cell count to ensure optimal seeding density.
- Remove the coating solution and add an appropriate volume of medium to the culture vessel. Do not let the culture vessel dry out.
- Plate the resuspended cells drop-wise and evenly at a density of 150,000-200,000 cells/cm^2.
- The day after plating, replace all of the medium with fresh pre-warmed, 37°C, Motor Neuron Maintenance Medium (without Y-27632 2HCl).

Differentiation and Maintenance of Human iPSC-Derived Motor Neurons

- To maintain a healthy culture, replace half the volume of medium every other day with fresh pre-warmed, 37°C, Motor Neuron Maintenance supplemented with CNTF (10 ng/mL) BDNF (5 ng/mL) and retinoic acid (0.5 μM).
- Human iPSC-derived Motor Neurons should be cultured for a minimum of 19-35 days to observe protein expression of HB9, ChAT and ISL-1.
- Repetitive firing of action potentials can be observed from 6-7 weeks in culture.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at
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Or
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