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Document # INST-FP-6051 05/12
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Human Motor Neuron Progenitor Cell Systems

hMNPs – Instructions for Use

Introduction

Human Motor Neuron Progenitors (hMNPs) are derived from human embryonic stem cells (hESCs). Each cryovial will yield ≥4 x 10^6 viable hMNPs. The recommended seeding density is approximately 120,000 viable cells/cm^2 on Poly-D-Lysine with Laminin coated plates. A small percentage of cells may proliferate.

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells: Remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.

Using media or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

Coating Plates

Human Motor Neuron Progenitors need an appropriate substrate to adhere and survive. The preferred substrate is Poly-D-Lysine with Laminin. For coating plates with Poly-D-Lysine with Laminin, do the following:

1. Prepare a solution of 50 µg/mL high molecular weight Poly-D-Lysine (Sigma) in HBSS or CTS (Cell Transplant Solution, California Stem Cell, Inc., Irvine, CA).
2. Using pipette tips, add 100 µL of the Poly-D-Lysine solution per well of a 96-well plate, 0.5 mL per well of a 24-well plate, 1 mL per well of a 12-well plate, 1 mL per well of a 6-well plate, 750 µL per well of a 6-well plate, or 250 µL per well of an 8-well slide.
3. Incubate the plate overnight at 37ºC in the dark, then aspirate the unbound Poly-D-Lysine solution using a sterile pipette.
4. After incubation, wash plates with an equal amount of sterile water for injection and allow the plates to air-dry in the dark for a minimum of 30 minutes. Ensure plates are completely dry and proceed to Laminin coating.
5. Prepare a solution of 15 µg/mL Laminin (Roche or Trevigen) in NeuroBlast™ Media.
6. Using pipette tips, add 75 µL of the Laminin solution per well of a 96-well plate, 0.3 mL per well of a 24-well plate, 750 µL per well of a 12-well plate, 2 mL per well of a 6-well plate, 750 µL per well of a 6-well plate, or 250 µL per well of an 8-well slide.
7. Incubate the plate at 37ºC, in the dark, for a minimum of 1 hour and a maximum of 6 hours.
8. Aspirate the unbound Laminin solution using a sterile pipette immediately before seeding cells.

Thawing of Cells / Initiation of Culture Process

1. The recommended seeding density for hMNPs is 120,000 cells/cm^2 (or 40,000 cells/well of a 96-well plate) on plates coated with Poly-D-Lysine/Laminin.
2. Warm 20 ml of NeuroBlast™ Medium to 37℃ in a sterile container.
3. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, and then retighten. Quickly thaw the cryovial in a 37℃ water bath being careful not to submerge the entire vial. Watch your cryovial closely; when the last sliver of ice melts, remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
4. Gently add 1.0 ml of the pre-warmed NeuroBlast™ Medium, one drop at a time into the vials of cells while rotating the tube by hand. This should take approximately two minutes. **Important:** do not add the whole volume of medium at once to the cells.
5. Resuspend the cells in the cryovial and using a micropipette, transfer the cell suspension into a 15 mL conical tube containing 9 mL of pre-warmed NeuroBlast™ Medium.
6. Centrifuge at 200 rcf (~1200 rpm) for 5 minutes at room temperature.
7. Resuspend the pellet in 10 mL of pre-warmed NeuroBlast™ Medium by gently pipetting up and down.
8. Count the total number of viable cells using the Trypan Blue dye exclusion method.
9. Use the following equation to determine the total number of viable cells.

$$\text{Total \# of Viable Cells} = \frac{\text{Total cell count} \times \text{percent viability}}{100}$$

10. Use the following equation to determine the total number of viable cells per ml.

$$\text{Total \# of Viable Cells per ml} = \frac{\text{Total \# of Viable Cells}}{10\,\text{ml}}$$

Plating of Cells into a 96-Well Plate

NOTE: Alternative plating formats can be used so long as cells are plated at 120,000 viable cells/cm^2 on plates coated with Poly-D-Lysine/Laminin and containing an appropriate volume of media for the vessel being used. The instructions below are specifically for plating cells into a 96-well plate. Alternative plating formats would require a modification of the protocol as appropriate.

1. Use the following equation to determine the total volume of cell suspension needed to plate a 96-well plate.

$$\text{Volume Needed (in ml)} = \frac{4\times10^6\ \text{Viable Cells}}{\text{Total \# of Viable Cell per ml}}$$

2. In a separate 50 mL conical tube, add the calculated volume of cell suspension needed and add enough NeuroBlast™ Medium to obtain a final volume of 30 mL.
3. On pre-coated Poly-D-Lysine and Laminin coated 96-well plate, seed 300 µL of the cell suspension per well using a multi-channel pipette or a liquid handler for a final concentration of 40,000 viable cells per well of the 96-well plate.
4. Incubate the cells at 37℃ with 5% CO_2.
5. The plate can be observed the day after plating.

Maintenance after Plating for a 96-Well Plate

1. Change 50% the medium 48 hours after seeding and 50% of the medium every other day thereafter as needed.
2. Warm 15 ml of MotorBlast™ Medium per every 96-well plate to 37℃ in a sterile container in the incubator.
3. Remove 50% (150 µL) of media from each well, being careful not to aspirate the cells attached to the bottom of the wells or touch the bottom of wells with the pipette.
4. Replace the removed media by slowly adding 150 µL of fresh, pre-warmed MotorBlast™ Medium to each well, and return plate to incubator.
5. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.

Quality Control

All donors are screened for HIV-1, Hepatitis B and Hepatits C. Cell lots are routinely tested to be free of bacteria and mycoplasma. MNPs are inspected for the morphology, adherence, aggregation and density and characterized using immunocytochemical staining. MNPs express over 75% TUJ1 (Neuronal Class III β-Tubulin) and HB9 (Homeobox Transcription Factor) and less than 15% of GFAP (Glial Fibrillary Acid Protein). Certificates of Analysis (COA) for each cell strain are shipped with

each order. COAs for all other products are available upon request.

Ordering Information

Cryopreserved Human Motor Neuron Progenitor Cells:

Cat. No.	Product	Description
FP-6051	hMNP	≥4 x 10^6 cells

Human Motor Neuron Progenitor Cell Media (Sold Separately):

Cat. No.	Product	Description
FP-6072	NeuroBlast™ Medium	50 ml NeuroBlast™ (plating medium)
FP-6025	MotorBlast™ Medium	100 ml MotorBlast™ (culture medium)
FP-6059	MotorBlast™ Medium	500 ml MotorBlast™ (culture medium)

Related Products

Plated Human ESC Derived Progenitor Cells:

Cat. No.	Product	Description
FP-6011	Standard MotorPlate™ Motor Neuron Progenitors (96-well plate)	Plated Human ESC Motor Neuron Progenitors in a 96-well plate
FP-6046	Mature MotorPlate™ Motor Neuron Progenitors (96-well plate)	Plated Human ESC Mature Motor Neuron Progenitors in a 96-well plate

Refer to the Lonza website or contact Scientific Support for details. Sample plates also available.

Product Warranty

Cultures have a finite lifespan *in vitro.*

California Stem Cells, Inc. guarantees the performance of its cells only if the recommended media and reagents are used exclusively, and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete cell system.

When placing an order or for Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Lonza Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, request additional information, or to speak with Scientific Support, you may contact Lonza by web, e-mail, telephone, fax or mail (See page 1 for details).

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

WARNING: CALIFORNIA STEM CELL, INC PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th ed. If you require further information, please contact your site safety officer or Scientific Support.