User Guide

Quick-Neuron™ Precursor - Human iPSC-derived Neural Precursor Cells

Catalog Numbers: NP-mRNA-CW50065, NP-mRNA-CW10149, NP-mRNA-CW20300, NP-mRNA-CW50023, NP-mRNA-CW70067, NP-mRNA-CW50025, NP-mRNA-CW50113, NP-mRNA-CW50114, NP-mRNA-CW50115, NP-mRNA-CW50137, NP-mRNA-CW50147, NP-mRNA-CW60130, NP-mRNA-CW60231, NP-mRNA-CW60236, NP-mRNA-CW20026, NP-mRNA-CW20090, or NP-mRNA-CW10130

Introduction

Elixirgen Scientific's proprietary transcription factor-based technology allows rapid and reproducible differentiation of human induced pluripotent stem cells (hiPSCs) into neurons without sacrificing the purity of the cells. Our Quick-Neuron™ Precursor - Human iPSC-derived Neural Precursor Cells (NPCs) continue to proliferate and express a variety of NPC markers, such as neuroectodermal stem cell marker nestin (NES) and vimentin (VIM). When handled and maintained according to the instructions in this user guide, NPCs are viable long-term and are suitable for further characterization and differentiation.

Scale: Quick-Neuron™ Precursor - Human iPSC-derived Neural Precursor Cells are available in two sizes: (Small) 1 million viable cryopreserved cells and (Large) 5 x 1 million viable cryopreserved cells. The instructions outlined in this user guide are for seeding 1 million viable cells into 6 wells of a 6-well plate (1.2×10^5 cells/well or 1.25×10^4 cells/cm^2).

Related Products: Quick-Neuron™ Precursor - mRNA Kit (Small), Catalog Number: NP-mRNA-S

Kit Contents

Upon receipt, immediately store the item as indicated. Be especially careful to keep the frozen cells on dry ice until placing them in liquid nitrogen and avoid any temperature fluctuation and slight thawing.

Contents	Amount (Small Size)	Amount (Large Size)	Storage
Cryopreserved cells	>1 million viable cells, (1 vial, 500 µl)	5 x >1 million viable cells, (5 vials, 5 x 500 µl)	Liquid nitrogen

Conditions of Use

This product is for research use only. It is not approved for use in humans or for therapeutic or diagnostic use.

Technical Support

For technical support, please contact us at cs@elixirgensci.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

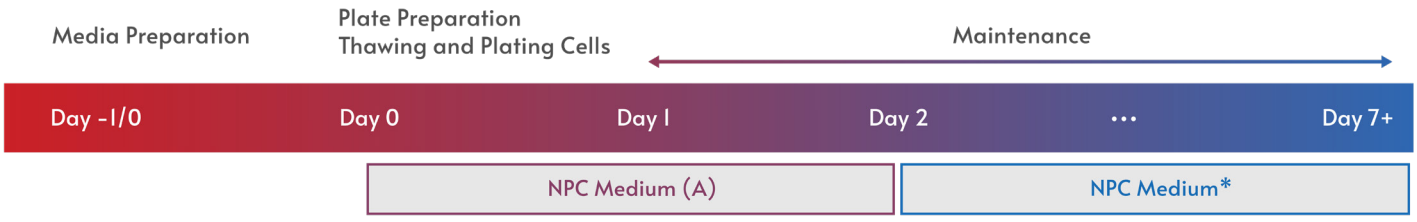
Last revised: April 29, 2021

Required Consumables

Item	Vendor	Catalog Number
6-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-80
DMEM/F12	ThermoFisher	21331020
Neurobasal Medium	ThermoFisher	21103049
N2 supplement (100x)	ThermoFisher	17502001
B-27 Supplement (50X)	ThermoFisher	17504001
Glutamax (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
Recombinant Human FGF-basic (154 a.a.) (bFGF)	PeproTech	100-18B
Recombinant Human HB-EGF (EGF)	PeproTech	100-47
LDN 193189 dihydrochloride	R&D Systems	6053
SB 431542	R&D Systems	1614
Poly-L-Ornithine*	Sigma-Aldrich	P4957-50ML
Extracellular Matrix such as* • Laminin Mouse Protein, Natural, or • Geltrex Basement Membrane Matrix	ThermoFisher	23017015 or A15696-01
TrypLE Select Enzyme (1X)**	ThermoFisher	12563011
0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
Phosphate-buffered saline (without Ca^{++} Mg^{++})	ThermoFisher	20012050
Bovine Serum Albumin solution	Sigma-Aldrich	A9576-50ML
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418
(Optional) STEM-CELLBANKER***	AMSBIO	11890

* Can be substituted with our Neuron Coating Solutions (Coating Materials B and C), Catalog Number: NCS.
** Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.
*** This is only required if you intend to cryopreserve the cells after thawing and expanding.

Workflow



*From Day 7, users may continue to maintain NPCs in NPC Medium to repeat this cycle or freeze cells down for future use.

Media Preparation

0.002% Poly-L-Ornithine solution (ornithine)*

1. Take 700 µl 0.01% Poly-L-Ornithine solution and mix it with 2.8 ml PBS.
2. Store the resulting 0.002% Poly-L-Ornithine solution, hereafter referred to as ornithine, for up to 2 weeks at 4°C.

*Ornithine can be substituted with Coating Material B from our Neuron Coating Solutions, Catalog Number: NCS.

1 mg/ml laminin stock solution (laminin)*

1. Thaw Laminin Mouse Protein, Natural and chill PBS at 4°C or on ice.
2. Mix the Laminin Mouse Protein, Natural and PBS to make the 1 mg/ml stock solution, hereafter referred to as laminin.
    - Laminin concentration varies by lot, so use the number specified on the vial or CoA when making your calculations.
3. Make aliquots of a convenient volume (e.g., 35 µl) and store at -20°C.

*Laminin can be substituted with Coating Material C from our Neuron Coating Solutions, Catalog Number: NCS.

0.5X TrypLE Select with EDTA (Solution D1)**

1. Mix 1.5 ml TrypLE Select Enzyme (1X) with 1.5 ml 0.02% EDTA in DPBS.
2. This mixture, hereafter referred to as Solution D1, can be stored at 4°C for 2 weeks.

**Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

10 µg/ml bFGF stock solution

1. Prepare 1 mg/ml bFGF by following the vendor's instructions.
2. Further dilute with sterile 0.1% BSA prepared with PBS to 10 µg/ml bFGF stock solution.
3. Make aliquots of a convenient volume (e.g., 100 µl).
4. This solution, hereafter referred to as 10µg/ml bFGF, can be stored at -80°C.

100 µg/ml EGF stock solution

1. Prepare 1 mg/ml EGF by following the vendor's instructions.
2. Further dilute with sterile 0.1% BSA prepared with PBS to 100 µg/ml EGF stock solution.
3. Make aliquots of a convenient volume (e.g., 20 µl).
4. This solution, hereafter referred to as 100µg/ml EGF, can be stored at -80°C.

10 mM LDN-193189 stock solution

1. Dissolve 10 mg LDN-193189 in 1.99 ml DMSO.
2. Make aliquots of a convenient volume (e.g., 100 µl).
3. This solution, hereafter referred to as 10mM LDN-193189, can be stored at -20°C.

1 mM LDN-193189 stock solution

1. Dilute 10 µl 10mM LDN-193189 in 90 µl DMSO.
2. Make aliquots of a convenient volume (e.g., 10 µl).
3. This solution, hereafter referred to as 1 mM LDN-193189, can be stored at -20°C.

10 mM SB 431542 (1,000x) stock solution

1. Dissolve 10 mg SB 431542 in 2.38 ml DMSO.
2. Make aliquots of a convenient volume (e.g., 100 µl).
3. This solution is hereafter referred to as 10mM SB 431542 and can be stored at -80°C.

NPC Medium

1. Prepare NPC Medium using the reagents listed in the table below. The volume below is sufficient for use from thawing cells to a day before the first passaging at Day 7.
    - Thaw N2 supplement (100x), B-27 Supplement (50x), 10 µg/ml bFGF, and 100 µg/ml EGF on ice for 20-30 minutes.
    - Thaw all other reagents at room temperature for 20-30 minutes.
    - Store NPC Medium for up to 2 weeks at 4°C. The leftover reagents can be saved for other uses.

NPC Medium Reagents	Volume
DMEM/F12	34.6 ml
Neurobasal Medium	34.6 ml
200 mM Glutamax (100x)	360 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	720 µl
N2 supplement (100x)	1.44 ml
B-27 Supplement (50X)	720 µl
10 µg/ml bFGF	144 µl
100 µg/ml EGF	14.4 µl

NPC Medium (A)

1. Prepare NPC Medium (A) using the reagents listed in the table below.
    - Warm/thaw all reagents at room temperature for 20-30 minutes.
    - **IMPORTANT!** Prepare NPC Medium (A) the day of thawing and plating the cells.

NPC Medium (A) Reagents	Volume
NPC Medium	36 ml
1 mM LDN-193189	3.6 µl
10 mM SB431542 (1,000x)	36 µl

Day 0

5-6 hr

Plate Preparation

IMPORTANT! Cells can be plated on a variety of plate formats including glass coverslips and chamber slides. Here each of the following steps assumes the use of 6 wells of a 6-well plate, with a surface area size of 9.5 cm². Other well formats can be used by adjusting medium volume proportionally to the surface area size.

1. Warm NPC Medium (A) and thaw ornithine* at room temperature for 20-30 minutes.
2. Vortex ornithine briefly and centrifuge it at a maximum speed for 1 minute.
3. Add 1.5 ml ornithine to the surface of each well.
4. Incubate the plate at 37°C, 5% CO_2 for at least 2 hours (or at 4°C overnight one day before plating).
5. Thaw laminin* and chill 3.3 ml PBS on ice for 20-30 minutes.
6. Add 33 µl laminin to the chilled PBS. Mix well.
    - All PBS washes should be done dropwise and with room temperature PBS. Chilled PBS is only for the coating step.
7. Aspirate the supernatant from each well and add 2 ml PBS.
8. Repeat Step 7.
9. Aspirate PBS from each well and add 1.5 ml diluted laminin.
10. Incubate the plate at 37°C, 5% CO_2 for at least 2 hours or until cells are ready for plating.
11. After the laminin incubation, pipet out most of the supernatant, but not all, from each well using a P1000 pipettor.
12. Rinse with 2 ml PBS.
13. Pipet out most of the PBS, but not all, from each well.
14. Repeat Steps 12-13.

15. Add 500 µl NPC Medium (A) to each well.
16. Incubate the plate at 37°C, 5% CO_2 for at least 2 hours or until cells are ready for plating.

*Ornithine and laminin can be substituted with Coating Materials B and C, respectively, from our Neuron Coating Solutions, Catalog Number: NCS.

Thawing Cells

1. Warm NPC Medium (A) at room temperature for 20-30 minutes.
2. Take out the vial of frozen cells from the liquid nitrogen storage tank.
3. Incubate the cryovial in a water bath set at 37°C (do not submerge the cap) until most of the content is thawed but a small ice crystal remains (~2 min).
4. Wipe the vial with a dry paper towel. Spray the vial with 70% ethanol and place it inside a biosafety cabinet.
5. Transfer 4.5 ml room temperature NPC Medium (A) to a new 15 ml conical tube.
6. Set a P1000 pipettor to 1 ml but take approximately 500 µl NPC Medium (A) from the 15 ml conical and add it to the cryovial dropwise at 1 drop per 1-2 seconds.
    - **IMPORTANT!** Use the same pipette tip for Steps 6-10.
7. Gently pipet the cell suspension up and down once.
8. Gently transfer all of the cell suspension to the 15 ml conical tube prepared in Step 5.
9. Take 1 ml of the cell suspension from the conical tube and add it to the original cryovial and pipet up and down 2-3 times and then transfer the whole contents back to the same 15 ml conical tube.
10. Mix the contents in the conical tube by gently pipetting cell suspension up and down 3 times.
11. Centrifuge the cell suspension in the 15 ml conical tube at 200 x g for 4 minutes.
12. Use an aspirator to remove most of the supernatant from the conical tube, leaving a small volume of the supernatant (<50 µl) to cover the pellet.
13. Tap the side of the conical tube up to 10 times to break up the cell pellet.
14. Add 1 ml room temperature NPC Medium (A) to the conical tube and pipet up and down no more than 2-3 times.

Plating Cells

1. Count cells to determine the volume of cell suspension needed for 6 wells and include a 10% buffer for cell number and volume (a total of 7.9 x 10^5 cells to plate 1.2 x 10^5 cells in each of the 6 wells). Adjust the volume to 9.9 ml with NPC Medium (A). If the volume of the cell suspension needed to get 7.9 x 10^5 cells exceeds 9.9 ml, centrifuge the required volume of cell suspension at 200 x g for 4 minutes, remove the supernatant, and resuspend the pellet into 9.9 ml NPC Medium (A).
2. Add 1.5 ml cell suspension to each well.
3. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 1

 <1 hr

Maintenance

1. Warm NPC Medium (A) at room temperature for 20-30 min.
2. Pipet out the old medium from each well and add 2 ml PBS to each well along its wall.
    - **IMPORTANT!** To avoid lifting cells, leave a small volume of the old medium (i.e., just enough to cover the surface of the well). PBS should be applied along the wall of the well very slowly. This procedure should be followed during all subsequent medium changes (i.e., on Day 2 and Day 4).
3. Pipet out the PBS and add 2.5 ml NPC Medium (A) to each well along its wall.
4. Incubate the culture at 37°C, 5% CO_2 overnight.

Day 2

 <1 hr

Maintenance

1. Warm NPC Medium at room temperature for 20-30 min.
2. Pipet out the old medium from each well and add 2 ml PBS to each well along its wall.
3. Pipet out the PBS and add 2.5 ml NPC Medium to each well along its wall.
4. Incubate the culture at 37°C, 5% CO_2 for 2 days.

Day 4

<1 hr

Maintenance

1. Warm NPC Medium at room temperature for 20-30 min.
2. Pipet out the old medium from each well and add 2 ml PBS to each well along its wall.
3. Pipet out the PBS and add 3 ml NPC Medium to each well along its wall.
4. Incubate the culture at 37°C, 5% CO_2 for 3 days.

Day 7

5-6 hr

If you intend to cryopreserve cells at this point, skip “Plate Preparation” and follow only the “Passaging Cells” section below and then proceed to the Appendix for freezing instructions.

Plate Preparation

Follow the instructions provided in the “Plate Preparation” section on Day 0 to prepare the number of wells and the plate format best suited for your purposes. In step 15 of plate preparation, when replacing the PBS with medium, use NPC Medium.

Passaging Cells

IMPORTANT! For the following steps, gently pipet and add solutions. Differentiating cells are delicate and should be handled with great care.

1. Warm Solution D1 at room temperature for at least 1 hour before use.
2. Working one well at a time, pipet out the old medium from each well and add 1 ml PBS and gently rock the plate.
3. Working one well at a time, pipet out the PBS from each well and add 300 µl Solution D1.
4. Rock the plate 3 times to spread the Solution D1 evenly.
5. Incubate the cultures at 37°C, 5% CO_2 for 3 minutes.
6. Working one well at a time, gently pipet out Solution D1 from each well and add 750 µl NPC Medium to each well along the wall of the well.
7. Working one well at a time, disperse the medium quickly over the bottom surface of the well by pipetting 6-8 times to detach cells using a P1000 pipettor.
8. Observe cells and/or cell aggregates floating in the well under a microscope. It is normal that 10-20% of cells remain attached to the well bottom after pipetting. The clusters of cells are not supposed to be lifted. Do not attempt to detach all of the cells remaining on the well bottom.
9. Gently pipet the cell suspension up and down in the well up to 5 times to break the cell aggregates using a P1000 pipettor. Excessive pipetting can damage the already-suspended neuronal cells.
10. Collect all of the cell suspension from each well in a tube using the same P1000 pipette tip.

Plating Cells

Follow the instructions provided in the “Plating Cells” section on Day 0 to plate cells in the number of wells and the plate format best suited for your purposes. Use NPC Medium.

Days 8-14

Maintenance

Follow the instructions provided for Day 1-7, while replacing the culture medium with NPC Medium. Continue following these instructions to maintain the cells, passaging or freezing them approximately every 7 days when they reach confluency, until they stop proliferating.

Appendix

Freezing Cells Down

After thawing frozen cells, approximately 50% of cryopreserved cells will be recovered as viable cells.

1. Determine the volume of the cell suspension and number of cryovials needed to freeze 0.2 ~ 2 x 10^6 cells per cryovial.
2. Centrifuge at 200 x g for 4 min.
3. While waiting for the centrifugation, label each cryovial. We recommend writing the name of the NPC lot number used, the passage number, the type of cells, the harvesting day and date, and the number of cells in the vial.
4. Aspirate the supernatant and resuspend the pellet with 0.5 ml / vial STEM-CELLBANKER.
5. Distribute 0.5 ml of the suspension to each cryovial.
6. Make sure that the caps are closed tightly and transfer the cryovials into a Mr. Frosty Freezing Container. Make sure that Mr. Frosty contains 250 ml isopropanol.
7. Loosely close the lid of Mr. Frosty with cryovials, put it into a -80°C freezer and leave it overnight or up to a few days.
8. Transfer the cryovials into a liquid nitrogen storage tank.

Appendix

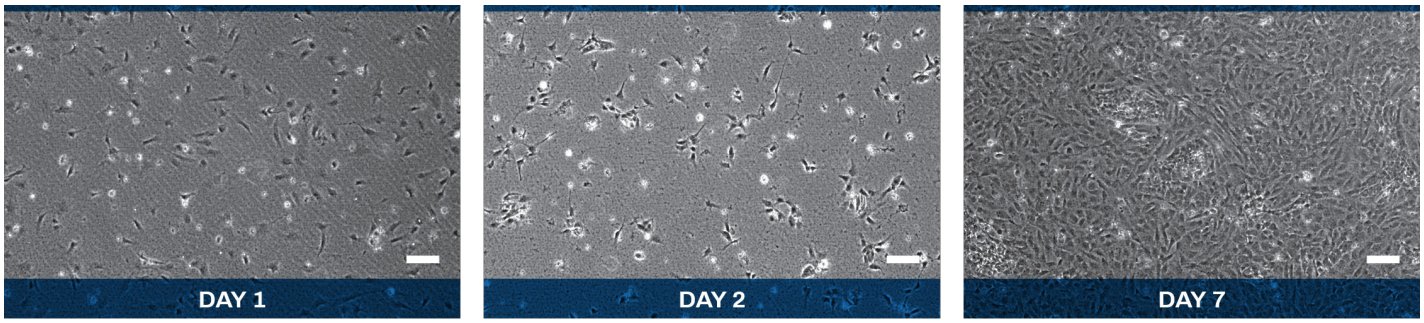


Figure 1. Representative phase contrast images of Quick-Neuron™ Precursor - Human iPSC-derived NPCs on days 1-7 post-thaw (scale bar = 100 µm). Cells are ready for passaging at Day 7.

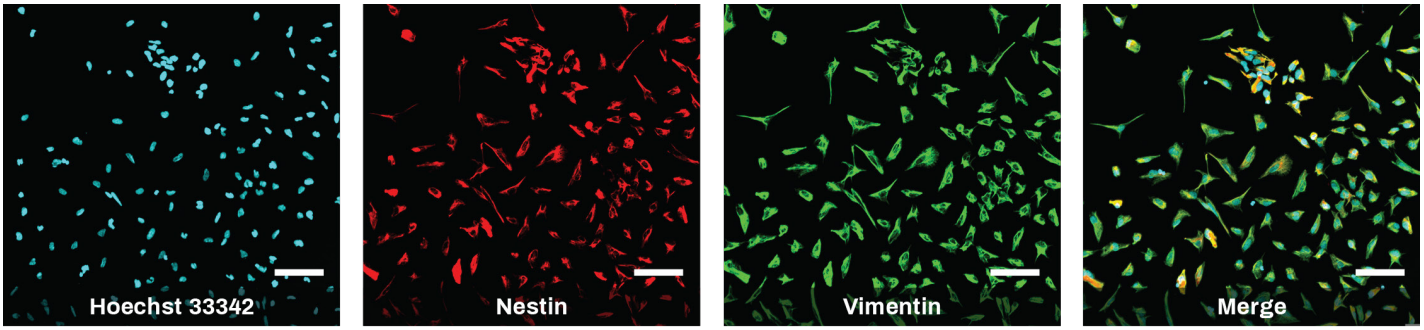


Figure 2. Immunofluorescent staining of Quick-Neuron™ Precursor - Human iPSC-derived NPCs show expression of nestin and vimentin on day 13 post-thaw (scale bar = 100 µm). Staining conditions: Anti-Nestin Antibody, clone 10C2 (Millipore Sigma, Catalog Number: MAB5326, 1:500 dilution) was used in combination with a secondary antibody (Invitrogen, Catalog Number: A11032 Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, 1:500 dilution). Recombinant Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (abcam, Catalog Number: ab92547, 1:500 dilution) was used in combination with a secondary antibody (Invitrogen, Catalog Number: A32731 Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488, 1:500 dilution). Nuclei were counterstained with Hoechst 33342.