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 stem cell differentiation method uses synthetic mRNAs to produce highly pure populations of neurons without a genetic footprint. Quick-Neuron™ Precursor - Human iPSC-derived Neural Precursor Cells continue to proliferate and express a variety of NPC markers such as nestin (NES), vimentin (VIM), Hes Family BHLH Transcription Factor 5 (HES5), SRY-Box Transcription Factor 1 (SOX1), and SRY-Box Transcription Factor 2 (SOX2). 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 at approximately 5 x 10^4 cells/cm^2 into 2 wells of a 6-well plate (5 x 10^5 cells/well), 10 wells of a 24-well plate (1 x 10^5 cells/well), or 62 wells of a 96-well plate (1.6 x 10^4 cells/well).

Related Products: Quick-Neuron™ Precursor - mRNA Kit, Catalog Number: NP-mRNA
Quick-Neuron™ Precursor - Maintenance Medium, Catalog Number: NP-MM

Contents

Upon receipt, immediately store the items at the indicated temperatures. 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	Thaw
Cryopreserved cells	>1 million viable cells, (1 vial, 500 µl)	5 x >1 million viable cells, (5 vials, 5 x 500 µl)	Liquid nitrogen	37°C

Condition 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 refer to the FAQ on our website.
You may also contact us at cs@elixirgensci.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

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
Gibco™ B-27™ Supplement (50X), minus vitamin A	Fisher Scientific	12-587-010
Gibco™ N-2 Supplement (100X)	Fisher Scientific	17-502-001
Glutamax (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
Recombinant Human FGF-basic (154 a.a.) (bFGF)	PeproTech	100-18B
Recombinant Human HB-EGF	PeproTech	100-47
LDN 193189 dihydrochloride	R&D Systems	6053
SB 431542	R&D Systems	1614
Bovine Serum Albumin solution, 30% in DPBS	Sigma-Aldrich	A9576-50ML
TrypLE Select Enzyme (1X)	ThermoFisher	12563011
0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
0.01% 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
Phosphate-buffered saline (without Ca^{++} Mg^{++})*	ThermoFisher	20012050
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D2650
(Optional) STEM-CELLBANKER**	AMSBIO	11890

*PBS should be used at room temperature unless otherwise specified.
**This is only required if you intend to cryopreserve the cells after differentiation.

Workflow

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

Media Preparation

0.002% Poly-L-Ornithine solution (ornithine)

1. Take 1 ml 0.01% Poly-L-Ornithine solution and mix it with 4 ml PBS.
2. The 0.002% Poly-L-Ornithine solution, hereafter referred to as ornithine, can be stored at 4°C for up to 2 weeks.

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.

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.

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 10 µg/ml bFGF, can be stored at -80°C.

100 µg/ml HB-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 100 µg/ml HB-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, hereafter referred to as 10mM SB 431542, can be stored at -20°C.

NPC Medium

1. Prepare NPC Medium (A) using the reagents listed in the table below. The volume below is sufficient for use from thawing cells to a day before first passaging at Day 7.

- Thaw N2 supplement (100x), B-27 Supplement (50x), 10 µg/ml bFGF, and 100 µg/ml HB-EGF on ice for 20-30 minutes.
- Warm all other reagents at room temperature for 20-30 minutes.
- Briefly spin down all tubes before use.
- Store NPC Medium for up to 2 weeks at 4°C. The leftover reagents can be saved for other uses.

Reagents	Volume
DMEM/F12	17.3 ml
Neurobasal	17.3 ml
200 mM GlutaMAX	180 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	360 µl
N2 supplement (100x)	360 µl
B-27 Supplement (50X)	720 µl
10 µg/ml bFGF	72 µl
100 µg/ml HB-EGF	7.2 µl
1 mM LDN-193189	2.2 µl
10 mM SB431542 (1,000x)	22 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.
   - Briefly spin down all tubes before use.
   - **IMPORTANT!** Prepare NPC Medium (A) the day of thawing and plating the cells.

Reagents	Volume
NPC Medium	18 ml
1 mM LDN-193189	1.8 µl
10 mM SB431542 (1,000x)	18 µl

Day 0

5-6 hours

Plate Preparation

IMPORTANT! Cells can be plated in 6-well, 24-well, and 96-well plates depending on the desired format. Refer to the table at the bottom of this page for the recommended volumes per well.

1. Vortex ornithine briefly and centrifuge it at a maximum speed for 1 minute.
2. Add ornithine to each well in the volume specified in the table.
3. Incubate the plate at 37°C, 5% CO_2 for at least 2 hours (or at 4°C overnight one day before plating).
4. Thaw laminin and chill PBS on ice for 20-30 minutes in the volumes calculated in the table. Add 1/100 volume of laminin to chilled PBS. Mix well.
   - All PBS washes should be done dropwise and with room temperature PBS. Chilled PBS is only for the coating step.
5. Aspirate the supernatant from each well and add PBS in the volume specified in the table.
6. Repeat Step 5.
7. Aspirate PBS from each well and add diluted laminin in the volume specified in the table.

8. Incubate the plate at 37°C, 5% CO_2 for at least 2 hours.
9. While the plate is incubating, prepare Medium iN(S1P) using the volume of Medium N(S1P) indicated in the table calculated for the number of wells in use plus 1.1 ml for resuspension (i.e., 2 wells of a 6-well plate needs 3.3 ml Medium N(S1P) (1.1 x 2 + 1.1), 10 wells of a 24-well plate needs 4.4 ml Medium N(S1P) (0.33 x 10 + 1.1) for this step).
    - Thaw iROCK at room temperature for 20-30 minutes.
    - Add 1/1000 of iRock to Medium N(S1P).
    - Keep the rest of Medium N(S1P) at 4°C for later use.
10. After the laminin incubation, aspirate most, but not all, of the supernatant and add PBS in the volume specified in the table. Add the PBS dropwise to each well.
11. Pipet out most of the PBS, but not all, from each well.
12. Repeat Steps 10-11.
13. Add Medium iN(S1P) to each well in the volume specified in the table.
14. Incubate the plate at 37°C, 5% CO_2 until cells are ready for plating.

Recommended volumes per well for different plate formats.

Reagents	Corresponding steps	Recommended volume per well		
		6-well plate	24-well plate	96-well plate
Ornithine	1, 2	1.5 ml	300 µl	50 µl
PBS for laminin dilution	4	1.65 ml	330 µl	55 µl
PBS	5, 6, 10, 11	2 ml	500 µl	100 µl
Diluted laminin	7	1.5 ml	300 µl	50 µl
Medium N(S1P) for Medium iN(S1P)	9	1.1 ml	330 µl	55 µl
Medium iN(S1P)	13	500 µl	200 µl	33 µl

Thawing Cells

1. Warm Medium N 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 Medium N to a new 15 ml conical tube.
6. Set a P1000 pipette to 1 ml but take approximately 500 µl Medium N 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 Medium iN(S1P) to the conical tube using a P1000 pipettor and pipet up and down no more than 2-3 times.

Plating Cells

1. Count the cells to determine the volume of cell suspension needed for the chosen number of wells and include a 10% buffer for cell number and volume (e.g., for a 24-well plate scenario, a total of 1.1 x 10^6 cells to plate 1 x 10^5 cells in each of the 10 wells). If the volume of the cell suspension needs to be adjusted, centrifuge the required volume of cell suspension at 200 x g for 4 minutes, remove the supernatant, and resuspend the pellet with Medium iN(S1P) to reach the multiplied volume of cell suspension with the number of wells.
2. Add cell suspension to the center of each well. Since each well already has Medium iN(S1P), the total volume of the medium in each well is indicated in the table below.
3. Incubate at 37°C, 5% CO_2 overnight.

	Recommended amounts		
	6-well plate	24-well plate	96-well plate
Viable cells/well	5 x 10^5 cells	1 x 10^5 cells	1.6 x 10^4 cells
Required total volume of cell suspension • (Volume of cell suspension/well) + 10% buffer	550 µl	110 µl	19 µl
Volume of cell suspension distributed/well	500 µl	100 µl	17 µl
Total volume/well • Medium iN(S1P) + cell suspension	1 ml	300 µl	50 µl

Day 1

< 1 hour

Maintenance

1. Warm Medium N(S1P) at room temperature for 30 minutes.
2. Pipet out the old medium from each well and add PBS to each well along its wall according to the table below.
    - **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.

Required volume per well	6-well plate	24-well plate	96-well plate
PBS	2 ml	500 µl	100 µl

3. Pipet out the PBS from each well and add Medium N(S1P) to each well along its wall according to the table below.

Required volume per well	6-well plate	24-well plate	96-well plate
Medium N(S1P)	2 ml	500 µl	100 µl

4. Incubate at 37°C, 5% CO_2 for 2-3 days.

Day 3 or 4

< 1 hour

Maintenance

1. Warm Medium N(S1P) at room temperature for 20-30 minutes.
2. Pipet out half the old medium from each well and add Medium N(S1P) according to the table below.

Required volume per well	6-well plate	24-well plate	96-well plate

Medium N(S1P)	1 ml	400 µl	75 µl

3. Incubate at 37°C, 5% CO_2 for 2-3 days.

Day 6

< 1 hour

Assay or Continuous Maturation

- Differentiated neurons can be observed on Day 1. For more mature neurons, we recommend culturing cells until Day 6. From Day 6, users may maintain differentiated neurons in the medium best suited for their needs, though we recommend Quick-Neuron™ Sensory Maintenance Medium, Catalog Number: SS-MM
- Differentiation into sensory neurons after using Quick-Neuron™ Sensory - Human iPSC-derived Neurons can be confirmed with the sensory markers, PRPH, ISL1, and BRN3A.

Appendix

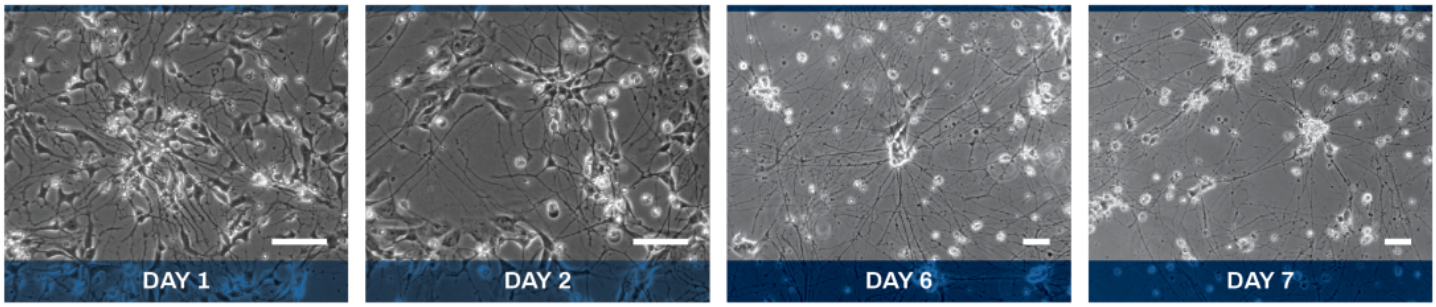

Figure 1. Representative phase contrast images of Quick-Neuron™ Sensory - Human iPSC-derived Neurons cell cultures on days 1, 2, 6 and 7 post-thaw (scale bar = 100 µm).

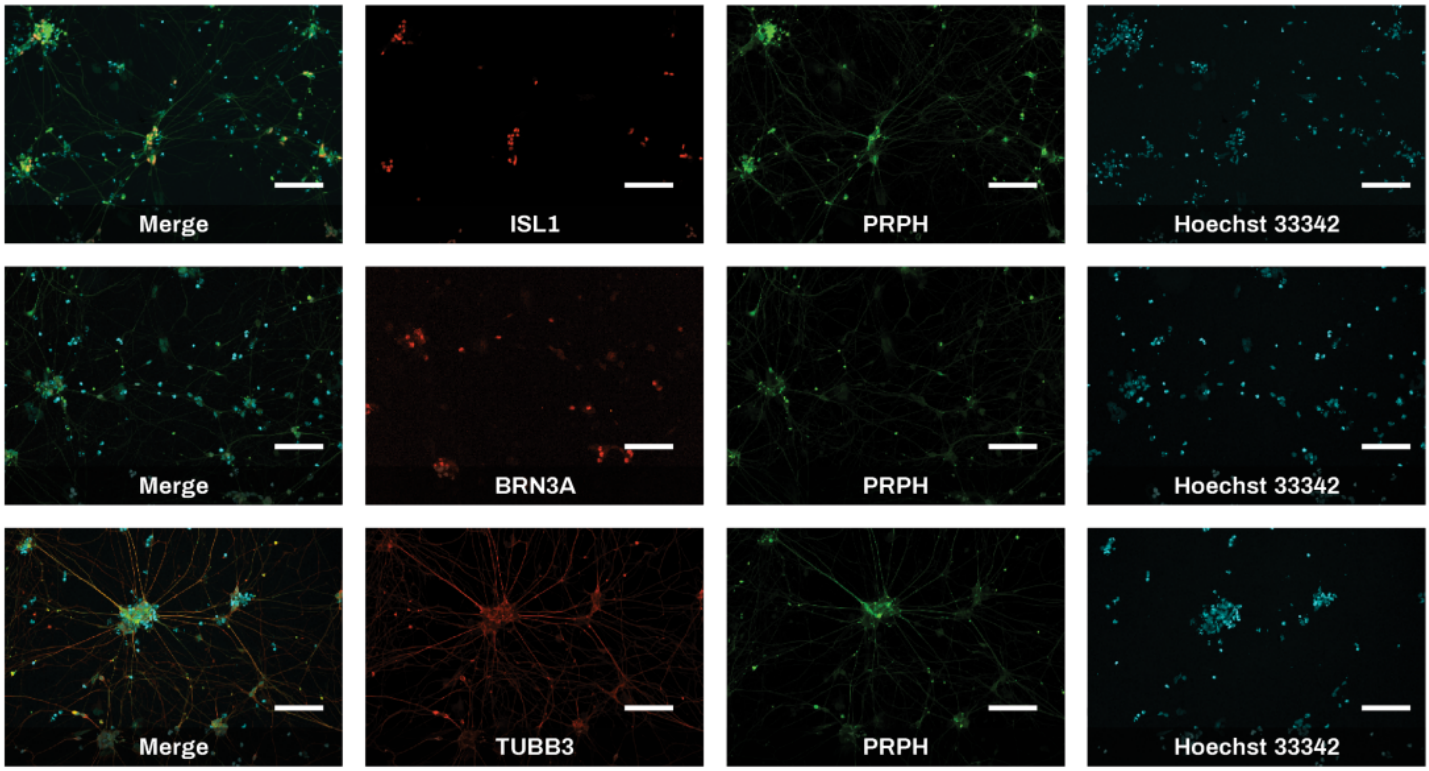

Figure 2. Immunofluorescent staining of Quick-Neuron™ Sensory - mRNA- CW Kit cell cultures shows typical neurite growth and expression of PRPH, TUBB3, BRN3A, and ISL1 on day 10 post-differentiation (scale bar = 100 µm). Staining conditions: Peripherin Rabbit polyclonal antibody (ThermoFisher, Catalog number: PA110018, 1:2000 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). Anti-β-III tubulin monoclonal antibody (R&D Systems, Catalog Number: MAB1195, 1:250 dilution) 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). BRN3A(14A6):mouse antibody (Santa Cruz Biotechnology, Catalog number:sc-8429 ,1:200 dilution) 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). ISL1 (DSHB, Catalog number: 39.4D5, 1:200 dilution) antibody 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). Nuclei were counterstained with Hoechst 33342.