Rev. 1

Human Induced Pluripotent Stem Cell-derived Neural Stem Cells (HiPSC-NSC)

Catalog #1650

Cell Specification

Human Induced Pluripotent Stem Cell derived Neural Stem Cells (Cat. #1650) from ScienCell Research Laboratories are differentiated from a human induced pluripotent stem cell line (HiPSC), which is generated using mRNA reprogramming technology from Human Fibroblasts (HF). The monolayer HF-HiPSCs are efficiently converted to neural epithelium using HPSC Neural Induction Medium (PSCNIM, Cat. #5931), a serum-free medium for rapid and efficient neural induction of human pluripotent stem cells (hPSCs). Synergistic inhibition of glycogen synthase kinase 3 (GSK3) and transforming growth factor β (TGF-β) differentiates HiPSC to homogenous neural stem cells (NSC) within 7 days.

The derived NSC are characterized by immunofluorescence with antibodies specific to Nestin and SOX2. The cell population is highly pure: >95% of cells express Nestin and >95% of cells are SOX2 positive. HiPSC-NSC are cryopreserved at P0 and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. Cells are negative for mycoplasma, bacteria, yeast and fungi. After reviving, NSC can be maintained in HPSC Neural Induction Medium as an adherent culture. NSC are multipotent and able to differentiate into various neuronal and glial subtypes. Specific patterning cues, such as SHH, retinoic acid and FGF8, can be added after reviving to direct the cells to different neural lineages. To differentiate the HiPSC-NSC, medium containing specific growth factors should be used (not provided).

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1650	1	HiPSC-NSC	1mL	Liquid Nitrogen
5931	1	Neural Induction Medium-basal (PSCNIM)	50mL	4°C
5982	1	Neural Induction Medium Supplement (50X)	1mL	-20°C

Recommended Medium

It is recommended to use the provided Neural Induction Medium (PSCNIM, Cat. #5931) for plating HiPSC-NSC and expanding them in the short term. Adding ROCK inhibitor Y-27632 in the first 24 hours after reviving improves cell viability and attachment in adherent cultures.

Additional Materials Recommended (Not provided)

Cat. #	Product	Vendor
3432-005-01	Cultrex Basement Membrane Extract (BME)	R&D Systems
5803	StemDS® Human Pluripotent Stem Cell Dissociation Solution	ScienCell Research Laboratories
0303	DPBS without Ca^{2+} and Mg^{2+}	ScienCell Research Laboratories
1254	ROCK Inhibitor Y-27632	Tocris Bioscience

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Product Use

HiPSC-NSC are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer Cat. #1650 from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments. Store Cat. #5931 at 4°C and Cat. #5982 at -20°C.

Shipping

Cat. #1650 and Cat. #5982 are shipped on dry ice. Cat. #5931 is shipped at room temperature.

References

[1] Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. (2009) "Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling." *Nat Biotechnol*. 27(3): 275-280.

[2] Li W, Sun W, Zhang Y, Wei W, Ambasudhan R, Xia P, Talantova M, Lin T, Kim J, Wang X, Kim W, Lipton SA, Zhang K, Ding S. (2011) "Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors." *PNAS*. 108(20): 8299-8304.

Instructions for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return them to culture as quickly as possible with minimal handling!

***Note**: HiPSC-NSC are very sensitive cells and they can proliferate multiple times before becoming terminally differentiated if cells are grown using the following protocol. The following procedures are optimized for 12-well plates; if using a different plate type, adjust volumes accordingly.*

Initiating the culture as an adherent culture:

1. Prepare Cultrex BME-coated 2-3 wells of 12-well plate according to the manufacturer's instructions and warm to room temperature before using.
2. Prepare complete Neural Induction Medium (PSCNIM): thaw the 50x supplement at room temperature; decontaminate the external surfaces of medium bottle and supplement tube with 70% ethanol and transfer them to a sterile field. Aseptically open the supplement tube and add to the basal medium with a pipette. Rinse the tube with medium to recover the entire volume.

   Warm the medium to room temperature prior to thawing the cells. Prepare 11 mL of neural induction medium and transfer 10 mL of neural induction medium from 11 mL into the 15 mL of conical tube.
3. Take one vial of neural stem cells out of the liquid nitrogen. Immediately transfer the vial into a 37°C water bath and gently swirl it or until most of contents are thawed and only a small piece of ice remains.

   ***Note**: The viability of the cells will decrease if the vial contents are completely thawed.*
4. Immediately remove the vial from the water bath, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads. Using a 2 mL pipette, gently resuspend the contents of the vial. Transfer all suspension to 15 mL tube containing 10 mL of neural induction medium. Wash the emptied vial with 1 mL medium and combine with the cell suspension in the tube.

   ***Note**: Minimize the time for step 3-4.*
5. Centrifuge the tube at 1000 rpm for 5 minutes at room temperature.
6. Aspirate supernatant carefully. Be careful not to disturb the cell pellet.
7. Tighten the cap of the tube and loosen the cell pellet by tapping the bottom of the tube. Add 1 mL of the neural induction medium containing 5 µM ROCK inhibitor Y-27632 into the tube and mix well. If a large visible cell pellet is present, try to break them into small pieces by gently pipetting 2 – 3 times with a 2 mL pipette.

   ***Note**: Applying ROCK inhibitor Y-27632 in the first 24 hours improves the cell viability.*
8. Count cell number to seed the cells into each well.

   ***Note**: The recommended cell seeding density is 2.5 – 5 x 10^5 cells/cm^2.*

9. Bring the Cultrex BME coated plate to the hood and aspirate the Cultrex BME from the well. Add 1 mL of medium containing 5 µM ROCK inhibitor Y-27632 into the one well of 12 well plate. Apply desired cell suspension into each well. Replace the cover and gently rock the vessel to distribute the cells evenly.
10. Return the culture vessel to the incubator.
11. For best results, do not disturb the culture for 24 hours after the culture has been initiated. Change the medium the next day to remove unattached cells, then every other day thereafter.

Subculturing (Optional):

Note*: HiPSC-NSC can undergo multiple rounds of expansion and passaging while retaining their stemness. The following procedures are optimized for 6-well plates and indicated volumes are for one well of a 6-well plate.*

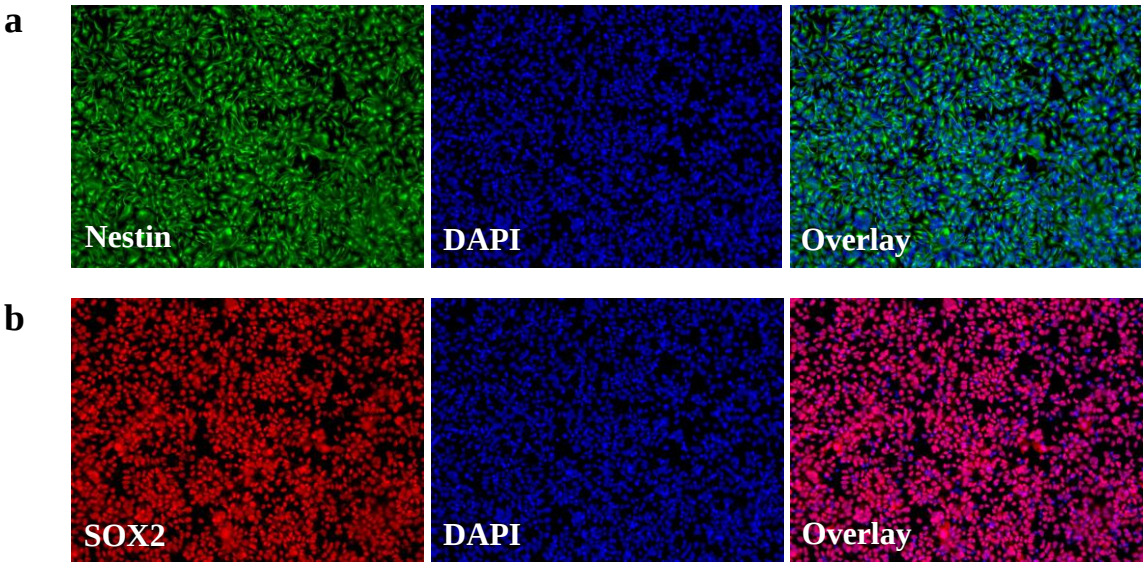
1. Subculture when the culture reaches 90% confluency.
2. Prepare Cultrex coated 6-well plate, StemDS® Human Pluripotent Stem Cell Dissociation Solution, complete Neural Induction Medium (PSCNIM), and DPBS (Ca^{2+} and Mg^{2+} free) and warm to room temperature
3. Prepare 5 mL of neural induction medium containing 5 µM ROCK inhibitor Y-27632 (not provided) and mix well.
4. Bring the Cultrex coated plate to the hood and aspirate the Cultrex from the well. Add 3 mL of medium prepared in step 3 into the well.
5. Bring the cells to the hood and aspirate medium. Rinse the cells with 2 mL of DPBS.
6. Aspirate DPBS and add 2 mL of StemDS® cell dissociation solution into the one well of 6-well plate containing cells for subculturing. Incubate the plate at 37°C incubator for 6-8 minutes. Do not incubate longer than 10 minutes in StemDS®. (Cells must be adherent during incubation with StemDS®).
7. Check cells under the microscope. Once cells completely round up, aspirate StemDS® Pluripotent Stem Cell Dissociation Solution and add 1 mL of medium prepared in step 3.
8. Gently resuspend cells several times with 1 mL pipette and transfer to a 15 mL conical tube. Repeat the procedure with another 1 mL of medium prepared in step 3 to collect residual cells on the plate.
9. Count cells. A seeding density of between 0.5-1 x 10^6 cells are recommended for each well.
10. Plate cells in a Cultrex coated 6 well plate containing 3 mL of medium. Replace the cover and gently rock the vessel to distribute the cells evenly.
11. Return the culture vessel to the incubator.

12. For best results, do not disturb the culture for 24 hours after the culture has been initiated. Change the medium the next day to remove unattached cells, then every other day thereafter.

Caution: Handling human-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

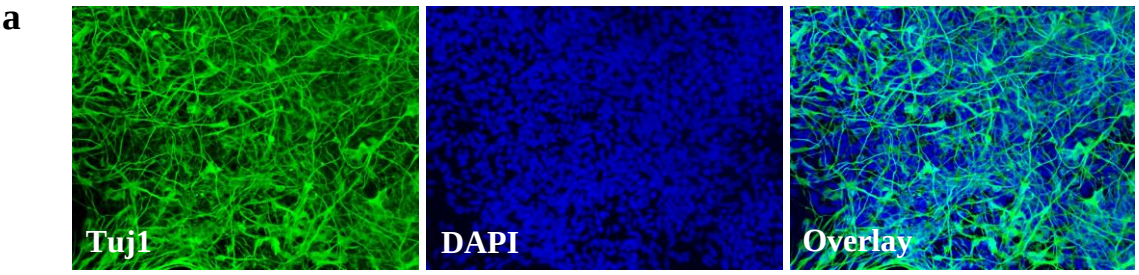
[1] Grizzle, W. E., and Polt, S. S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11(4).

Figure 1. Revived HiPSC-NSC express neural stem cell markers.



The revived HiPSC-NSC were characterized by immunostaining with antibodies against Nestin (a, green) and SOX2 (b, Red). Nuclei were stained with DAPI (blue). 100x

Figure 2. HPSC-NSC are able to differentiate into neurons.



HiPSC-derived NSC can differentiate into neurons (a, Tuj1, green) in Neural Differentiation Medium (NDM). 200x