Rev. 2

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). Dual modulation of Wnt and SMAD signaling pathway differentiates HiPSCs 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 Neural Expansion Medium (Cat. #5941) 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 (not provided), can be added after reviving to direct the cells to different neural lineages.

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1650	1	HiPSC-NSC	1mL	Liquid Nitrogen
5941	1	Neural Expansion Medium-basal (NEM)	100mL	4°C
5992	1	Neural Expansion Medium Supplement (50X)	2mL	-20°C

Recommended Medium

It is recommended to use the provided Neural Expansion Medium (NEM, Cat. #5941) 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
5813	CellEase 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. #1660 from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments. Store Cat. #5941 at 4°C and Cat. #5992 at -20°C.

Shipping

Cat. #1650 and Cat. #5992 are shipped on dry ice. Cat. #5941 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 37ºC 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.*

Initiating the culture as an adherent culture:

1. Prepare Cultrex BME-coated culture vessel according to the manufacturer's instructions for thin layer, non-gelling method, and warm to room temperature before using.
2. Prepare complete Neural Expansion Medium: 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 10 mL of Neural Expansion Medium containing 10 μM ROCK inhibitor Y-27632.

   ***Note**: Applying ROCK inhibitor Y-27632 in the first 24 hours improves the cell viability.*
3. Take one vial of neural stem cells out of the liquid nitrogen. Immediately transfer the vial into a 37ºC 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 homogenously. Keep a small sample for a later trypan blue viability cell count and transfer cell suspension to 15 mL tube containing 10 mL of Neural Expansion 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. Perform trypan blue viability cell count prior to plating
6. Bring the Cultrex BME coated culture vessel to the hood and aspirate the Cultrex BME coating solution. Gently mix cells to get a homogenous suspension with 5 mL pipette and seed the cells at 7-10 x 10^4 viable cells per cm^2. Cover and gently rock the vessel to distribute the cells evenly.
7. Return the culture vessel to the incubator at 37ºC 5% CO_2.
8. For best results, do not disturb the culture for 16 hours after the culture has been initiated. Change the medium the next day to remove unattached cells, then second or third day thereafter.

Subculturing (Optional):

***Note**: HiPSC-NSC can undergo multiple rounds of expansion and passaging while retaining their stemness.*

1. Subculture when the culture reaches ~90% confluency.
2. Prepare Cultrex coated culture vessels, CellEase Cell Dissociation Solution, complete Neural Expansion Medium, and DPBS (Ca^{2+} and Mg^{2+} free) and warm to room temperature
3. Prepare appropriate of Neural Expansion Medium containing 10 µM ROCK inhibitor Y-27632 (not provided) and mix well.
4. Bring the cells to the hood and aspirate medium. Rinse the cells with DPBS.
5. Aspirate DPBS and add CellEase Cell Dissociation Solution to sufficiently cover the cultureware surface. Incubate the culture vessel at 37°C incubator for 3-6 minutes.

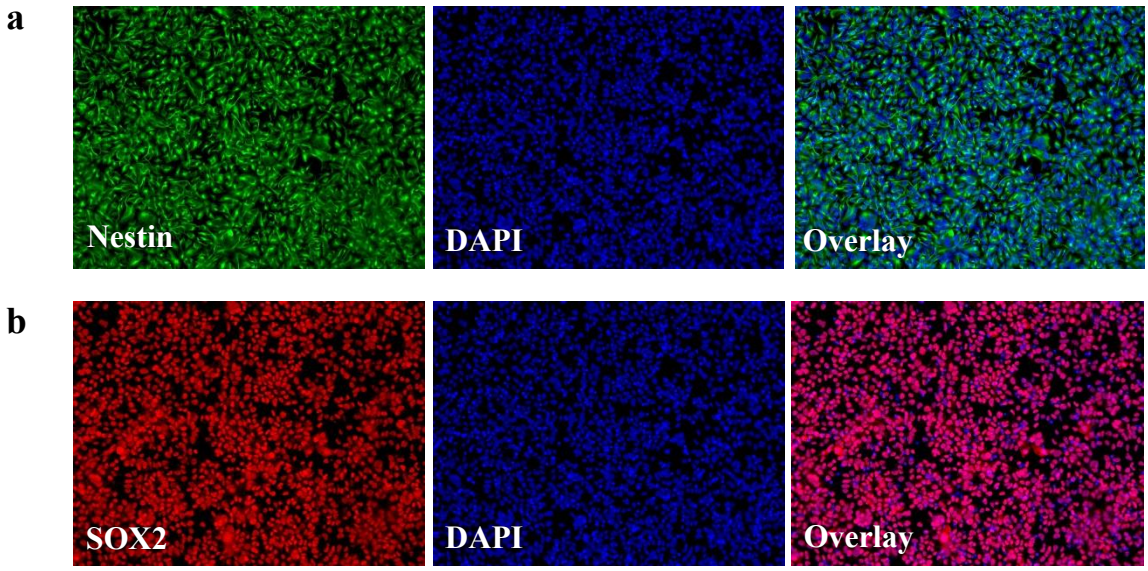
   ***Note**: High passage NSC might require longer incubation time.*

6. Check cells under the microscope. Once cells completely round up and detach upon tapping of the culture vessel, rinse the cells off gently with extra medium and transfer the cells to a conical tube for centrifugation.
7. Centrifuge the cells at 1000 rpm for 5 minutes. Gently resuspend cells in an appropriate volume of medium prepared in step 3.
8. Seed cells in a Cultrex coated culture vessel at 1:6 to 1:10 ratio. Rock the vessel to distribute the cells evenly.
9. Return the culture vessel to the incubator.
10. For best results, do not disturb the culture for 16 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].

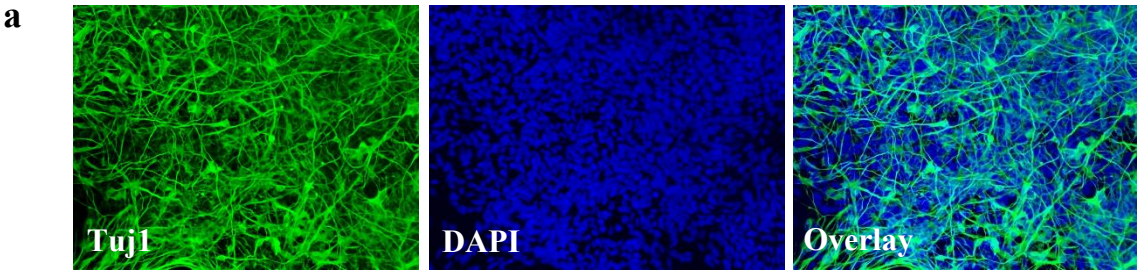
[1] Grizzle, W. E., and Polt, S. S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11(4).

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).

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



HiPSC-derived NSC can differentiate into neurons (a, Tuj1, green) in Neuronal Medium (Cat# 1521, NM).