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

Catalog #1640

Cell Specification

Human Induced Pluripotent Stem Cell derived Neural Crest Cells (Cat. #1640) 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 crest cells (NCC) using HPSC Neural Crest Medium (PSCNCM), 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), and activation of BMP4 signaling differentiates HiPSC to homogenous neural crest cells (NCC) within 7 days.

The derived NCC are characterized by immunofluorescence with antibodies specific to Sox10. The cell population is highly pure: >70% of cells express Sox10. HiPSC-NCC 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, NCC can be maintained in Neural Crest Cell Medium (NCCM) as an adherent culture for a short time. NCC are multipotent and able to differentiate into various cell types such as peripheral neurons, smooth muscle cells, melanocytes and so on. Specific factors can be added after reviving to direct the cells to terminal differentiation. To differentiate the HiPSC-NCC, medium containing specific growth factors and reagents should be used (not provided).

Product Content

Cat. #	# of vials	Product	Quantity	Storage
1640	2	HiPSC-NCC	1mL	Liquid Nitrogen
1641	1	Neural Crest Cell Medium-basal (NCCM)	100mL	4°C
1682	1	Neural Crest Cell Supplement (50X) (NCCS)	2mL	-20°C

Recommended Medium

It is recommended to use the provided Neural Crest Cell Medium (NCCM) for plating HiPSC-NCC and expanding them in the short term. Adding ROCK inhibitor Y-27632 in the first 12-18 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
0303	DPBS without Ca^{2+} and Mg^{2+}	ScienCell Research Laboratories
A3008	ROCK Inhibitor Y-27632	APExBIO

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Product Use

HiPSC-NCC 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. #1640 from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments. Store Cat. #1641 at 4°C and Cat. # 1682 at -20°C.

Shipping

Cat. #1640 and Cat. #1682 are shipped on dry ice. Cat. #1641 is shipped at room temperature.

References

[1] Bronner ME, LeDouarin NM. Development and evolution of the neural crest: an overview. Dev Biol. 2012 Jun 1;366(1):2-9. doi: 10.1016/j.ydbio.2011.12.042. Epub 2012 Jan 2. PMID: 22230617; PMCID: PMC3351559.

[2] Srinivasan A and Toh Y-C (2019) Human Pluripotent Stem Cell-Derived Neural Crest Cells for Tissue Regeneration and Disease Modeling. Front. Mol. Neurosci. 12:39. doi: 10.3389/fnmol.2019.00039

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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-NCC are very sensitive cells and they become terminally differentiated if cells are grown using specific growth factors and reagents. 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 six wells of 12-well plate according to the manufacturer's instructions and warm to room temperature before using.
2. Prepare complete Neural Crest Cell Medium (NCCM): 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.
3. Take two vials of neural crest 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 5 mL of Neural Crest Cell Medium (NCCM) with 10uM ROCK inhibitor. 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.*
5. Bring the Cultrex BME coated plate to the hood and aspirate the Cultrex BME from the well. Add 2 mL of Neural Crest Cell Medium (NCCM) containing 10uM ROCK inhibitor into each well of 12 well plate. Apply 1 mL of cell suspension into a well. Replace the cover and gently rock the vessel to distribute the cells evenly.
6. Return the culture vessel to the incubator.
7. For best results, do not disturb the culture for 12-18 hours after the culture has been initiated. Change the medium after 12-18 hours to remove unattached cells, then fix the cells for immunostaining or treat with medium containing specific growth factors and reagents (not provided) for terminal differentiation.

   ***Note**: As the purity of neural crest cells decreases over time, it is highly recommended that you plan ahead for your experiments.*

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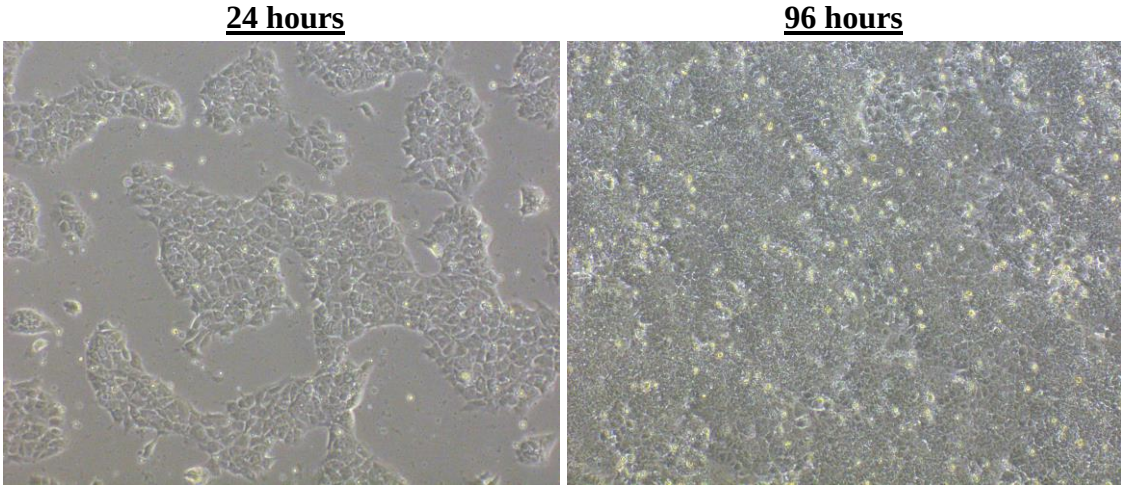
Caution: Handling human-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

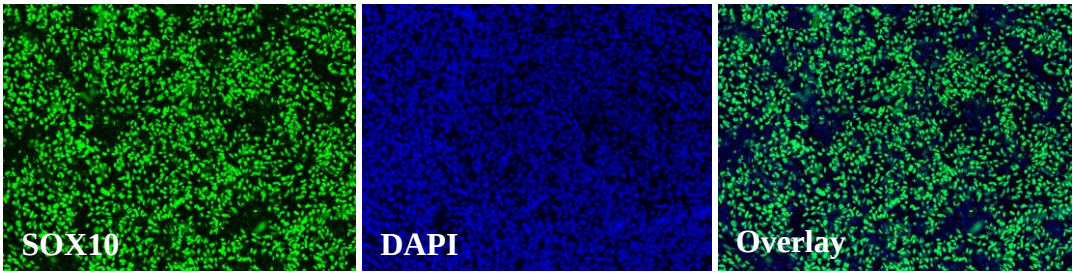
[1] Grizzle, W. E., and Polt, S. S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11(4).

Figure 1. HiPSC-NCC Differentiation at 24 hours and 96 hours of post-plating.



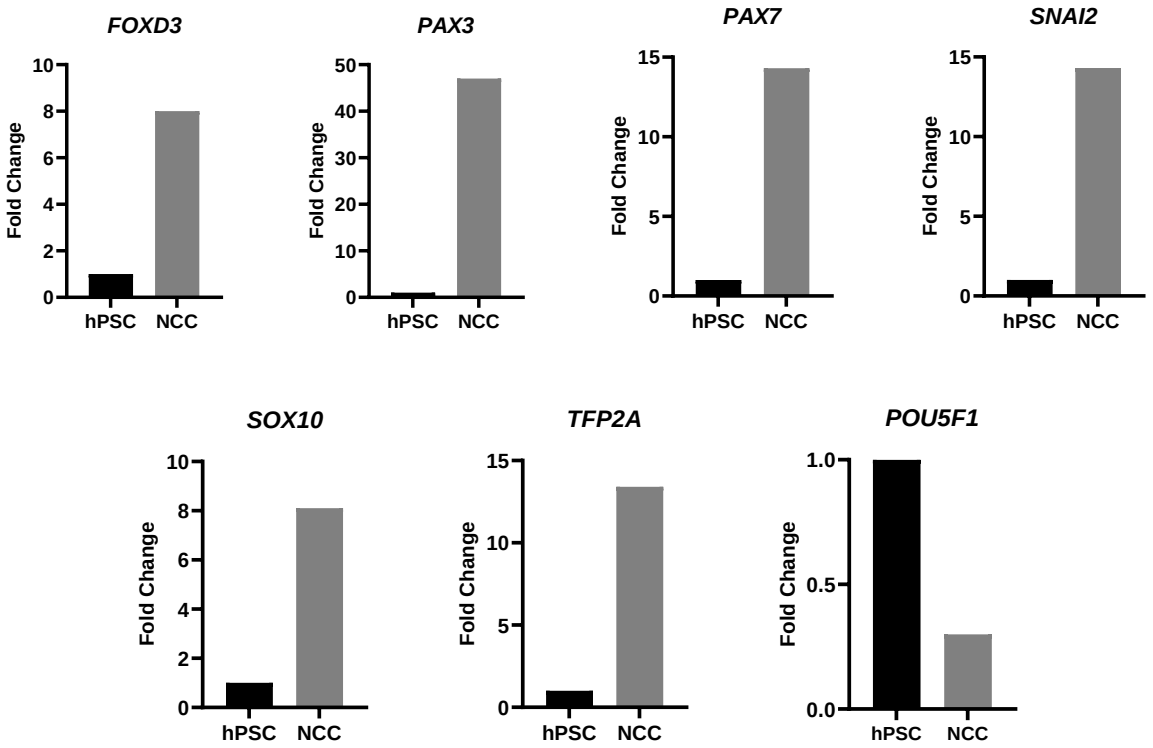
Pictures were taken 24 hours and 96 hours of post-plating. 100x

Figure 2. Revived HiPSC-NCC express neural crest cell markers: SOX10.



The revived HiPSC-NCC were characterized by immunostaining with antibody against SOX10 (green). Nuclei were stained with DAPI (blue). 100x

Figure 3. qPCR data representing change in expression of neural crest-associated genes: FOXD3, PAX3, PAX7, SNAI2, SOX10 and TFP2A.



The qPCR results demonstrate alterations in the expression of neural crest-associated genes FOXD3, PAX3, PAX7, SNAI2, SOX10 and TFP2A, and the stem cell marker OCT4 (POU5F1). Gene expression levels are normalized relative to the initial undifferentiated hPSCs.