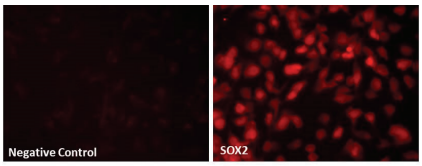
ANGIO-PROTEOMIE

DATA SHEET

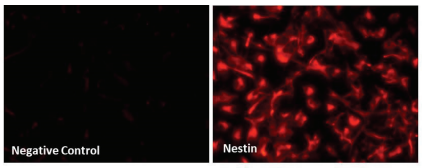
Human iPSC-Derived Neural Precursor Cells (iPSC-NPCs)

ORDER INFORMATION

Name of Cells: Human iPSC-Derived Neural Precursor Cells (iPSC-NPCs)
Catalogue Number: **cAP-0500-002-01**
Product Format: Cells in Frozen Vial
Cell Number: 1 x 10^6cells/vial



Human iPSC Derived NPCs are positive for SOX2 (hAP-5942)



Human iPSC Derived NPCs are positive for Nestin (hAP-1234)

General Information

Description

Human iPSC-Derived Neural Precursor Cells (iPSC-NPCs, cAP-0500-002-01) were differentiated from iPSCs (cAP-0500-002) using Neural Induction Medium (cAP-56). The cells are offered at passage 1, which are positive for Nestin, and SOX2. These cells can be expanded using Neural Precursor Medium (cAP-57).

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If, upon arrival, continued storage of the frozen vial is necessary, it should be stored in liquid nitrogen vapor phase, not at -80C. Storage at –80C will result in loss of viability.

1. Preparation for Cell Culture

1. Thaw iPSC Coating Solution (cAP-50) at room temperature. As soon the solution is thawed, then keep it at 4C to 8C (stable for 2 weeks).
2. The night before thawing the iPSC-NPCs – Prepare coated flask as described below.
3. Thirty minutes prior to handling the cells – Pre-warm Neural Precursor Medium (cAP-57) at 37C before adding to cells. If using ROCK Inhibitor Y27632 (cAP-52), prepare Neural Precursor medium supplemented with final concentration of 5uM ROCK Inhibitor Y27632. Neural Precursor Medium with ROCK inhibitor should be prepared freshly.

2. Protocol for Coating Flasks

Add 4ml of iPSC Coating Solution into 1 x T75 flask and leave the flask at 37C incubator for at least 1 hour (better for overnight). The flask is ready to be used once the excessive coating medium is aspirated (No need to be washed with PBS).

3. Initiation of Cultures

1. Rapidly thaw the cells by placing the cryovial in a 37C water bath, swirling gently. Remove the cryovial from the water bath when only a few ice crystals are remaining.
2. Sterilize the cryovial by rinsing with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Using a 1 mL pipette, gently transfer the cell suspension to a 15 mL conical tube.
4. Slowly add 5 mL Neural Precursor Medium (cAP-57) to the conical tube. Rinse the cryovial by adding and removing an additional 1 mL of medium and transfer the liquid to the 15 mL conical tube. Shake the conical tube gently to mix the cells while adding media.
5. Centrifuge the cells at 200 x g for 5 minutes.
6. Aspirate the supernatant and discard. Gently tap on the bottom of the tube to loosen the cell pellet.
7. Add 5ml of cAP-57 medium with ROCK Inhibitor Y27632 and gently resuspend the pellet by pipetting up and down 2 to 3 times.
8. Aspirate the iPSC Coating Solution from the flask prepared as in the Protocol for Coating flask section.

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9. Seed 10mL of suspended cells into the T75 flask prepared in step 8.
10. Incubate the culture at 37C in a suitable incubator. A 5% CO2 in air atmosphere is recommended.

Post thaw day 1, perform a 100% medium change and remove all cells that did not attach.

Perform a 100% medium change the day after cell inoculation, and then every other day. Passage the cells every 4 to 5 days (95% confluent) at an split ratio of 1:3.

ROCK Inhibitor Y27632 is not necessary each time the culture medium is changed. It is required when the cells are recovering from thaw or immediately after cells being passaged on iPSC Coating Solution coated flasks.

4. Subculturing Procedure

This protocol is designed to passage iPSC-NPCs cultured in a T75 flask, using iPSC Non-Enzymatic Dissociation Reagent (cAP-51) to detach the cells. The recommended spilt ratio is 1:3. Volumes should be adjusted according to the size and number of the tissue culture vessels to be processed.

1. Add 4ml of iPSC Coating Solution into each T75 flask (3 flasks are needed) and leave the coated flask at 37C for at least one hour (overnight preferred). The flasks are now ready for use.
2. Warm an aliquot of iPSC Non-Enzymatic Dissociation Solution to 37C
3. Aspirate and discard the culture medium.
4. Add 4ml of iPSC Non-Enzymatic Dissociation Solution to the flask.
5. Incubate at 37C for 2 to 5 minutes.
6. Add 4 mL of cAP-57 medium to the flask, and detach the cells by pipetting up and down 2 to 3 times with a 5 mL pipette.
7. Transfer the detached cells to a 15 mL conical tube.
8. Add an additional 3 mL of cAP-57 medium to the flask to collect any remaining cells. Transfer this rinse to the 15mL conical tube containing the detached cells.
9. Centrifuge the cells at 200 x g for 5 minutes.
10. Aspirate the supernatant and discard.
11. Add 12ml of cAP-57 medium with 5um ROCK inhibitor. Gently resuspend the cells by pipetting up and down **2 to 3 times** with a 10 mL pipette**.**
12. Add 4ml of cells suspension into each T75 iPSC Coating Solution coated flask containing 6mL cAP-57 (with 5um ROCK inhibitor)/T75 flask.
13. Incubate the culture at 37C in a humidified 5% CO2/95% air incubator. Perform a 100% medium change every other day (with the Medium without ROCK inhibitor thereafter). Passage the cells when cells are close to 95% confluent.

Cryopreservation

1. Detach NPSc from the T75 Flask as described in the recommended subculturing protocol (steps 1-10). Gently tap the bottom of the tube to loosen the cell pellet.
2. Take the iPSC Freezing Media (cAP-53) from storage and swirl to mix. Keep cold. Decontaminate by dipping in or spraying with 70% alcohol.
3. Add 3 mL of **cold** iPSC Freezing Media to the tube. Gently resuspend the pellet by pipetting up and down **2 to 3** times with a 1 mL tip.
4. Immediately transfer 1 mL each of the cell suspension into 3 (1 to 3 split ratio) labeled cryovials.
5. Freeze the cells gradually at a rate of 1C/min until the temperature reaches -70C to 80C.
6. The cells should not be left at 80 C for more than 48 hours. Once at 80 C, frozen cryovials should be transferred to the vapor phase of liquid nitrogen for long-term storage.

Use Restrictions

These cells are distributed for research purposes only.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

Warranty

The viability of our products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. We list the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the our recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, our warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While we use reasonable efforts to include accurate and up to date information on this product sheet, we make no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. We do not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. We are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, we are not liable for damages arising from the misidentification or misrepresentation of cultures.

Related products

iPSC SFM Culture Medium (Xeno-Free)	cAP-49	500ml	Angio-Proteomie
iPSC Coating Solution	cAP-50	50ml	Angio-Proteomie
iPSC Non-Enzymatic Dissociation Solution	cAP-51	50ml	Angio-Proteomie
ROCK Inhibitor (500 x) Solution	cAP-52	1 ml	Angio-Proteomie
iPSC Freezing Medium	cAP-53	50ml	Angio-Proteomie
Neural Induction Medium	cAP-56	100ml	Angio-Proteomie
Neural Precursor Medium	cAP-57	100ml	Angio-Proteomie

Caution: Handling human tissue derived products is potentially bio-hazardous. Although each cell strain is tested negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate; therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.