- NPC 凍管

1. IBMS-NPC-01-02 (P1)
2. IBMS-NPC-02-07 (P1)
3. IBMS-NPC-04-02 (P1)

- 鑑定資訊

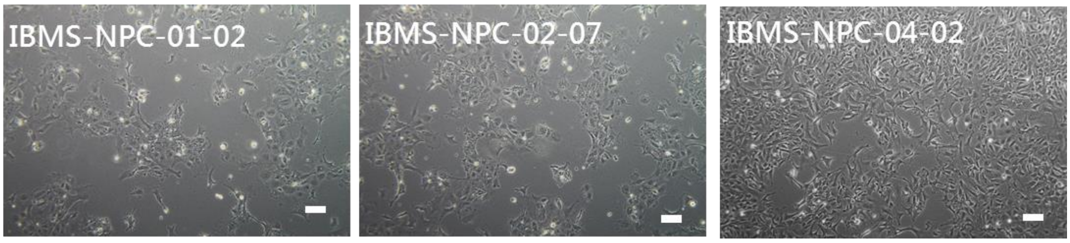
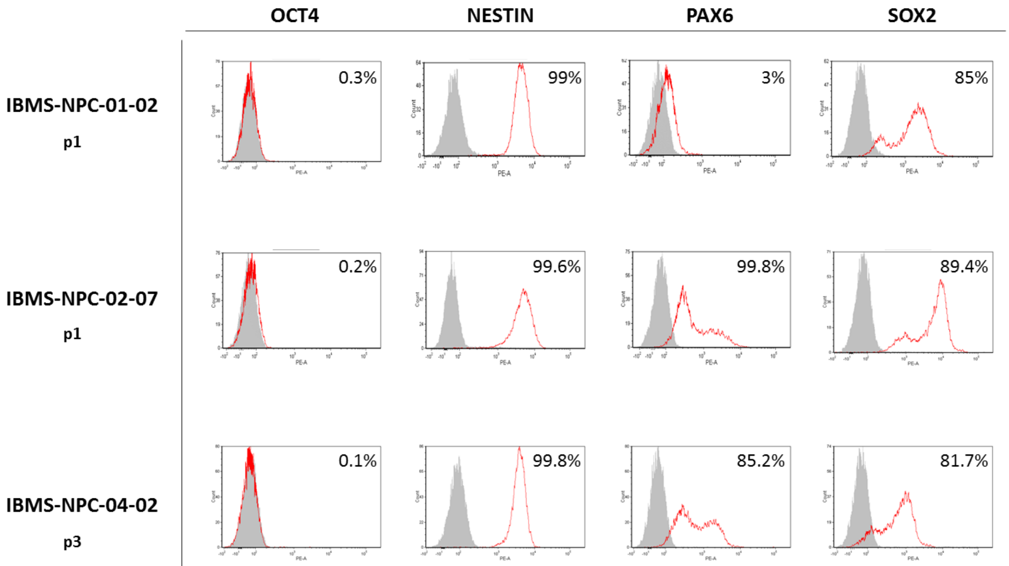


Figure 1. 細胞型態



Note: Please note that not all NSC will stain positive for PAX6, this is normal and to be expected (i.e., only NSCs that possess forebrain fate commitment will stain positive). In our experience ~15–50% of NSCs generated using PSC Neural Induction Medium stain positive for Pax6.

Figure 2. NPC markers

- 解凍培養與保存步驟

- **Neural Expansion Medium (for 100 mL)**

1. To prepare 100 mL of complete Neural Expansion Medium, aseptically mix the following components:

Neurobasal® Medium(Gibco, Cat. no. A1647801) 49 mL
Advanced™ DMEM/F-12(Gibco, Cat. no. 12634) 49 mL
Neural Induction Supplement(Gibco, Cat. no. A1647801) 2 mL

Note: Complete Neural Expansion Medium can be stored at 2–8°C in the dark for up to 2 weeks. Warm the Neural Expansion Medium in a 37°C water bath for 5–10 minutes before using.

- **ROCK Inhibitor Y27632 Solution (5 mM)**

1. To prepare 5 mM ROCK inhibitor Y27632 solution, aseptically mix the following components:
Y27632(Sigma-Aldrich, Cat. no. Y0503) 1 mg
Distilled water 0.625 mL

Note: After dissolving, filter through a 0.22 µm filter, aliquot 20–50 µL into sterile tubes, and store at –5°C to –20°C in the dark for up to 1 year. Thawed Y27632 solution can be kept at 4°C for up to 1 week.

- **Coat culture vessels with Geltrex® LDEV-Free, hESC-Qualified Basement Membrane Matrix**

1. Thaw a vial of Geltrex® LDEV-Free hESC-Qualified Reduced Growth Factor Basement Membrane Matrix(Gibco, Cat. no. A1413301 or A1413302) at
2–8°C overnight.
Note: Thawed Geltrex® matrix can be aliquoted and then frozen at –5°C to –20°C, or stored at 2–8°C for up to 2 weeks. Avoid repeated thawing and freezing.

2. To create a working solution, dilute the thawed Geltrex® matrix solution 1:100 with cold DMEM/F12 on ice.

3. Quickly cover the whole surface of each culture vessel with the Geltrex® matrix solution.
6-well plate (9.6 cm^2/well) → 1 mL/well
35-mm dish (11.8 cm^2)→ 1 mL
60-mm dish (20 cm^2)→2 mL

4. Incubate the culture vessels in a 37°C, 5% CO2 incubator for 1 hour.

5. The culture vessels are now ready for use. Just before use, aspirate the diluted Geltrex® solution from theculture vessels. Cells can be plated directly onto the Geltrex® matrix-coated culture vessels without rinsing.

Note: Coated culture vessels can also be stored at 2–8°C for up to one week. When storing, seal culture vessels with Parafilm® laboratory film to prevent drying. Before using, warm up the coated culture vessels stored at 2–8°C at room temperature for 30 minutes.

- **Recover cryopreserved NSCs**

1. Prepare Geltrex® matrix-coated dishes as previously described.

2. Remove cryotubes of NSCs from the liquid nitrogen storage tank using metal forceps.

3. Immerse the vial in a 37°C water bath without submerging the cap. Swirl the vial gently.

4. When only an ice crystal remains, remove the vial from the water bath.

5. Spray the outside of the vial with 70% ethanol and place it in the cell culture hood.

6. Pipet the cells gently into a sterile 15-mL conical tube using a 1-mL pipette.

7. Add 1 mL of DPBS into the vial to collect the resident cells. Transfer the DPBS from the vial and add it drop by-drop to the 15-mL conical tube. While adding, gently move the tube back and forth to mix the NSCs to reduce osmotic shock to cells.

8. Centrifuge the cells at 300 × g for 5 minutes and aspirate the supernatant.

9. Resuspend the cell pellet in DPBS. Centrifuge at 300 × g for 5 minutes and aspirate the supernatant.

10. Re-suspend the cell pellet in the appropriate amount (for example, 1 mL for all NSCs from 1 vial) of prewarmed Neural Expansion Medium.

11. Determine the cell concentration using your preferred method.

12. Dilute the cell suspension with pre-warmed Neural Expansion Medium into a solution containing $2 \times 10^5 – 4 \times 10^5$ cells/mL.

Note: The overnight treatment with the ROCK inhibitor Y27632 (final concentration of 5 μM) is required at the time of NSC plating to prevent cell death.

13. Aspirate the Geltrex® matrix solution from the Geltrex® matrix-coated vessels and add the appropriate amount of diluted cell suspension into each culture vessel to plate cells at the density of $0.5 \times 10^5 – 1 \times 10^5$ cells/cm^2.

6-well plate (9.6 cm^2/well) 2 mL/well
35-mm dish (11.8 cm^2) 2 mL
60-mm dish (20 cm^2) 3 mL

14. Move the culture vessels in several quick back-and-forth and side-to-side motions to disperse the cells across the surface, then gently place the vessels in the incubator.

Note: Avoid splashing the medium to the outsides of the well to avoid any possible contamination.

15. On day 2 of cell plating, exchange the Neural Expansion Medium. Continue to exchange the medium every other day until the NSCs reach confluency and are ready for further expansion.

Note: At day 1 after NSC plating, replace the medium with complete Neural Expansion Medium to eliminate Y27632 from the culture. Do not add ROCK inhibitor Y27632 during this step.

- **Cryopreserve NSCs**

1. Passage NSCs to at least P1 before cryopreservation.

2. When NSCs reach confluency, warm the appropriate amount of StemPro® Accutase® Cell Dissociation Reagent in a 37°C water bath.

3. Aspirate the spent medium and add pre-warmed StemPro® Accutase® reagent into culture vessels according

6-well plate	1 mL/well
35-mm dish	1 mL
60-mm dish	2 mL

4. Incubate for 3–5 minutes at 37°C until all cells detach from the surface of culture vessels.

5. Transfer the cells into a 15- or 50-mL conical tube using a 5-mL pipette.

6. Add the appropriate amount of DPBS to each well of the vessel (for example, add 1 mL to 1 well of a 6-well plate) to collect the residual cells, then add the cell suspension to the tube.

7. Triturate the cell suspension 3 times with a 5-mL or 10-mL pipette to break up the cell clumps.

8. Centrifuge the cells at 300 × g for 4 minutes and aspirate the supernatant.

9. Re-suspend the cells with the appropriate amount of DPBS and centrifuge the cells at 300 × g for 4 minutes.

10. Aspirate the supernatant and re-suspend the cells with the appropriate amount of Neural Expansion Medium.

11. Determine the cell concentration using your preferred method.

12. Dilute the cell suspension with Neural Expansion Medium to $2 \times 10^6 – 4 \times 10^6$ cells/mL.

13. Add the same volume of Neural Expansion Medium containing 20% DMSO.

14. Allocate 1 mL cell suspension into each cryotube and freeze cells at –80°C overnight in Nalgene® Mr. Frosty® Freezing Containers with isopropanol.

15. After freezing cells overnight, transfer the cells into a liquid nitrogen tank for long term storage.