hPSC Nucleofection Buffer

Product Manual

I. Product Introduction

Human pluripotent stem cells (hPSC), including human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC), are a class of stem cells capable of self-renewal, unlimited proliferation, and differentiation into almost all cell types in the human body. The hPSCs are a hotspot and focus of stem cell research. Our hPSC Nucleofection Buffer is specially developed for the electroporation of hPSCs cultured under various undifferentiated conditions. It achieves stablely high transfection efficiency, high post-transfection cell survival rates, is non-toxicity to the cells, and does not affect downstream experiments, making it the perfect solution for hPSC transfection.

II. Product Information

Table 1: Product description of hPSC Nucleofection Buffer

Product Information	Cat.No.	Amount
hPSC Nucleofection Buffer	RP01005	2.5 mL (25 rnx)

III. Storage Conditions

1. Storage temperature: 4°C.
2. Shelf life: 6 months.

IV. Equipment and Materials

Table 2: Recommended Equipment & Materials for hPSC Nucleofection

Human Pluripotent Stem Cell Nucleofection - Recommended Equipment and Materials
Electroporator: Amaxa Nucleofector (II, IIb or other models)
hPSC Nucleofection Buffer equilibrated to room temperature
Nucleofection cuvette and plastic dropper compatible with the electroporator
Plasmids for nucleofection (concentration 0.5-1 μg/μL, A260/A280 value around 1.80)
Suitable number of human pluripotent stem cells (2×10^6 - 4×10^6 cells per nucleofection reaction)
NcEpic or NcTarget pluripotent stem cell culture medium (or hPSC culture medium such as mTeSR, E8, etc.) and Matrigel- or Vitronectin-coated culture plates

V. Preparation before Nucleofection

1. hPSC culture (using ncEpic or ncTarget Pluripotent Stem Cell Medium/Matrigel culture system as an example)
   - hPSCs should be passaged at a ratio of 1:10-1:20, passaged every 4 days, and fresh culture medium should be replaced daily. It is recommended to hPSCs with a confluence of 80%-90% for electroporation.
2. hPSC Dissociation

   TIPS: Before nucleofection, hPSC need to be dissociated into single-cell suspension. Cell clumps can reduce transfection efficiency and post-nucleofection cell viability. You might need to optimize the dissociation protocol if the following steps do not result in a single-cell suspension on your cells.

   2.1. For hPSCs cultured in a feeder-free system (e.g., Matrigel), use pre-warmed Solase at room temperature to dissociate the cells in a 37°C incubator for 5-10 minutes to ensure full dissociation. Add pre-warmed DMEM/F12 medium to stop the dissociation, and pipette 1-2 times with a 5 ml pipette to obtain a single-cell suspension.

   2.2. For hPSCs cultured on a feeder system, passage them once and seed them onto Matrigel before the nucleofection experiment. Culture until the cells reach 80%-90% confluence of, then digest using the method described in section 2.1.

VI. hPSC Nucleofection

Table 3: Electroporation System

Taking a single well of a 6-well plate as an example, each nucleofection reaction system includes:
2×10^6 – 4×10^6 hPSCs
1–5 μg plasmid DNA
100 μL hPSC Nucleofection Buffer

1. Prepare the Amaxa Nucleofector II and set the Nucleofection program to B016.
2. Pre-warm the required volume of Solase (1 ml per well for a 6-well plate) and DMEM/F12 medium (3 ml per well for a 6-well plate) to room temperature.
3. Add 2 mL of culture medium + ROCK inhibitor to each well of the Matrigel-coated plate and place it in a 37°C incubator for pre-equilibration.
4. Add 1-5 μg of plasmid DNA into a sterile 1.5 mL centrifuge tube, ensuring the total volume does not exceed 10 μL.
5. Add 100 μL of hPSC Nucleofection Buffer to the 1.5 mL centrifuge tube containing the plasmid DNA, mix well. Briefly centrifuge to collect the mixture at the bottom of the tube.
6. Bring your hPSC culture to the biosafety cabinet, aspirate the culture medium, and rinse each well with 2 mL of DPBS and aspirate.
7. Add 1 ml of preheated Solase to each well and place the plate in a 37°Cincubator for 5-10 min for dissociation.
8. Add 2 mL of pre-warmed DMEM/F12 medium to each well and pipette 1-2 times to obtain a single-cell suspension.
9. Collect all cells into a 15 mL centrifuge tube and centrifuge at 120×g for 4 minutes.
10. Aspirate the supernatant from the 15 mL centrifuge tube and gently tap the bottom of the tube several times to loosen the cell pellet. Resuspend the cells in pre-warmed DMEM/F12 medium (approximately 1 mL per well) by pipetting 5-6 times.
11. Take 10 μL of the cell suspension for counting. Aliquot the cell suspension into new 1.5 mL centrifuge tubes at 2×10^6 - 4×10^6 cells per tube.

12. Briefly centrifuge the cells in a microcentrifuge for 8 seconds to collect them at the bottom of the tube. Carefully aspirate the supernatant and gently tap the bottom of the tube several times to loosen the cell pellet.
13. Take out the pre-equilibrated culture plate from step 3 and set it aside for use.

TIPS: All the following steps should be performed as quickly and gently as possible.

14. Add the prepared hPSC Nucleofection Buffer & plasmid cocktail from step 5 to each corresponding tube, and gently tap the bottom of the tubes several times to fully suspended the cells (Note: Avoid pipetting).
15. Transfer the cell-plasmid- Nucleofection buffer cocktail into the Nucleofection cuvette (note: pipette as gently as possible), tap the cuvette on the bench twice to remove bubbles, place it in the electroporator slot, and run Nucleofection program B016.
16. Remove the Nucleofection cuvette, draw approximately 500 µL of culture medium from the 6-well plate prepared in step 13, and slowly add it to the cuvette along the wall. Gently tap the cuvette twice on the bench to mix, then carefully transfer the mixture with a small pipette back into the corresponding wells of the 6-well plate. Gently rock the culture plate to ensure even cell distribution.
17. Place the culture plate back into the 37°C incubator. Gene expression can be assessed 16 - 24 hours post-nucleofection

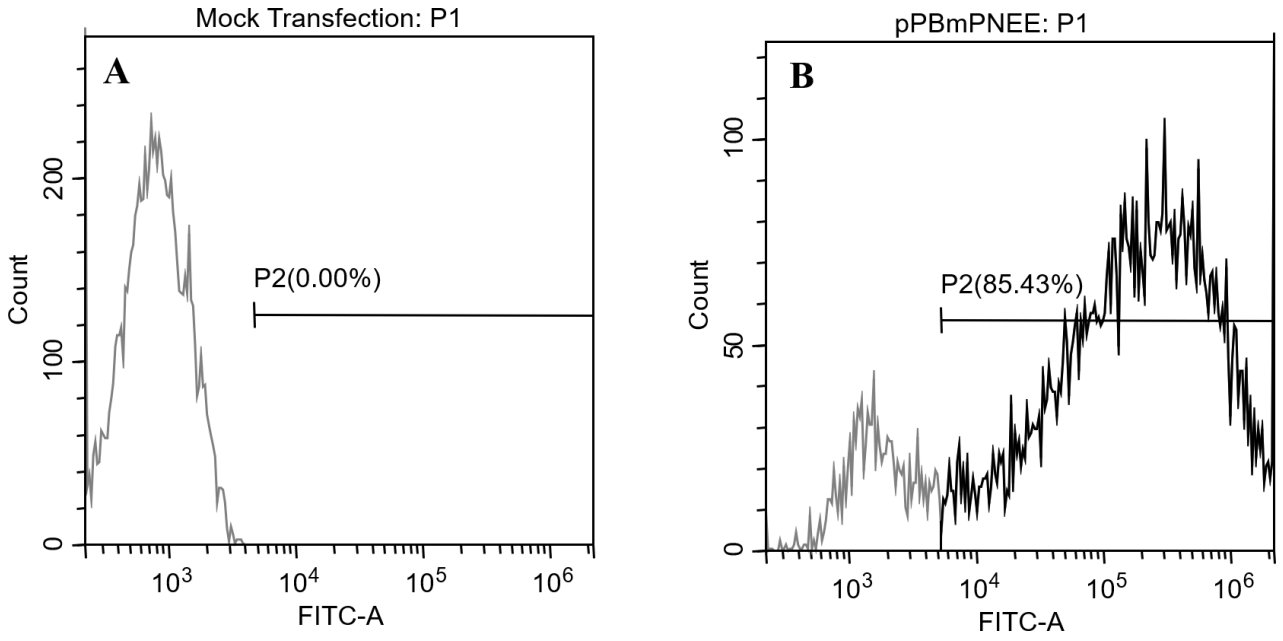

Figure 1: Example of hiPSC Transfection Using hPSC Nucleofection Buffer

2 µg of pPBmPNEE plasmid (expressing EGFP, ~6.5 kb) was nucleofected into hiPSCs.

24 hours post-nucleofection,EGFP-positive cells were detected by flow cytometry.

A: Negative control without plasmid during nucleofection.

B: pPBmPNEE plasmid added during electroporation. The transfection efficiency reaches 85.43%.