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NEPA21 Transfection of Cell Suspensions Using Cuvettes

Products

✓ NEPA21 electroporator
✓ CU500 Cuvette Chamber
✓ CU600 Cuvette Stand Holder
✓ EC-002S NEPA Electroporation Cuvettes, 2mm gap (a pipette is included with each cuvette)
After electroporation, when you pipette out cells from the cuvette:
please use the included pipette, or a commercially available gel loading tip. 
✓ EP buffer*: Opti-MEM medium (Invitrogen Sku# 31985-062, 31985-070 or 31985-088)
*We refer to this medium as “EP buffer” throughout this document.
NOTE: The serum-free and antibiotic-free Opti-MEM medium must be used.
✓ 6-well plates or culture plates of your choice for cell culture after electroporation
✓ Nepa Gene plasmid DNA (pCMV-EGFP) (1μg/μl in a tube)

Preparing Cells

1. Prepare cells according to the instructions of the supplier (optimal confluency: 70-80%).
2. Harvest the cells by trypsinization.
3. Neutralize trypsinization reaction with culture medium containing serum and supplements.
4. Centrifuge the cells.
5. Resuspend the cell pellet in the EP buffer enough to **wash the medium containing serum and supplements completely off the cells.**
6. Centrifuge the cells.
7. Resuspend the cell pellet in the EP buffer enough to **wash the medium containing serum and supplements completely off the cells.**
8. Centrifuge the cells.
9. Resuspend the cell pellet in the EP buffer.
10. Count the cells to determine the cell density.

NOTE: Transfection efficiency/viability will be severely reduced if any serum/antibiotic is left.
Pipette up and down repeatedly to achieve a monodisperse cell suspension without clumps of cells.

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Cells & DNA per Cuvette

The final density per cuvette: **1 x 10^6 cells and 10µg DNA in 100µl solution**.

- ✓ Cells: 1 x 10^6 cells in 90µl EP buffer
- ✓ Plasmid DNA: 10µg in 10µl solution (TE Buffer) of the DNA tube.

For 12 cuvettes,

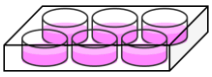
prepare 13 x (1 x 10^6 cells in 90µl EP buffer) and 13 x (10µg DNA in 10µl)

***If the number of the prepared cells is not enough, the cell number per cuvette can be decreased**, even though the transfection efficiency might be a little lower.

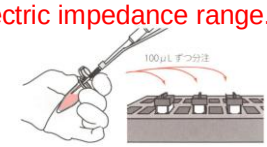
e.g.) 5 x 10^5 cells and 10µg DNA in 100µl solution per cuvette

Electroporation

1. Prepare culture plates by filling wells with culture media for post electroporation.

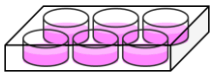


2. For 12 cuvettes, mix well 1.3x10^7cells in 1,170µl EP buffer and 130µg DNA in 130µl in a tube without foaming.
3. Dispense 100µl into each cuvette.
   The volume (100µl) should be as exact as possible, to achieve the same electric impedance range.



4. Set the electroporation parameters.
5. Mix the cells lightly without foaming for 1 second by tapping the cuvette with a finger.

6. Place the cuvette into the CU500 Cuvette Chamber.
7. Press the Ω button of NEPA21 and make a note of the impedance value.
   The range of the impedance value should be 30-55 Ω.
8. Press the Start button to execute the electroporation program.
9. Make a note of the values of currents and joules displayed in the Measurements frame.
10. Take the cuvette out of the Chamber.
11. Add some of the cell culture growth medium of step 1 into the cuvette using the included pipette.
12. Mix the cells and the medium in the cuvette well.



13. Completely pipette-out the sample from the cuvette and dispense it into the prepared well of step 1.
    NOTE: To avoid cell damage, RIGHT AFTER the electroporation, add the medium into the cuvette and remove the sample with the medium from the cuvette immediately.
14. Repeat the step 4-13.
15. Then incubate the cells.