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We use this protocol and it's working

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Cells electroporation for cell transfection with NEON system

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ABSTRACT

Protocol for cell electroporation with the NEON transfection system

1 Warm the resuspension buffer and the E2 buffer in the water bath out of the water at 37 °C

2 Put the electrode part inside the left hand side hood in 419

3 Put a NEON glass tube matching the electrode position

4 Add 3 mL of E2 buffer in the glass tube.

Note

The buffer should cover the electrode level

5 Switch on the NEON equipment

6 Load the saved settings.

Note

The settings depend on the cells used. I.e., we used 3 pulses of 10 ms at 1650 mV for C2C12 myoblasts

7 Add 5 µg of the plasmid in a sterile Eppendorf tube

8 Add 3 mL of fresh cell media without antibiotics in a T25 flask

9 Trypsinise the cells as usual

10 Once you have the cell pellet, discard the supernatant carefully

11 Wash the pellet with 150 µL of sterile DPBS and discard the supernatant carefully

12 Wash the cell pellet with 150 µL of resuspension buffer and discard the supernatant carefully

13 Resuspend the cell pellet with 130 µL of resuspension buffer and discard the buffer

14 Transfer 130 µL of the cell solution to the Eppendorf tube containing the plasmid

15 Mix without making any bubble

16 Take a 100 µL tip with the NEON pipette

17 Take 100 µL of the mixture with the NEON pipette and place it in the glass tube matching the electrode position

18 Press "START" in the NEON display

19 Once the "COMPLETE" message appears in the NEON display, put the cells with the NEON pipette in the T25 flask

20 Put the T25 flask in the “Antibiotics free” incubator

21 After 24:00:00 , check the cells under the microscope and perform the required experiment 1d