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Cells electroporation for cell transfection with NEON system

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Protocol status: Working

We use this protocol and it's working



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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 μ L of sterile DPBS and discard the supernatant carefully
- 12 Wash the cell pellet with  150 μ L of resuspension buffer and discard the supernatant carefully
- 13 Resuspend the cell pellet with  130 μ L of resuspension buffer and discard the buffer
- 14 Transfer  130 μ L of the cell solution to the Eppendorf tube containing the plasmid
- 15 Mix without making any bubble
- 16 Take a  100 μ L tip with the NEON pipette
- 17 Take  100 μ L 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

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