Nucleofection of Dissociated Hippocampal Neurons in Suspension

References

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Solutions

1. Calcium and magnesium-free Hank's balanced salt solution (CMF-HBSS): 1X HBSS (Invitrogen), 9.9 mM HEPES and 1 U/100 μg of penicillin/streptomycin in water. Store solution at 4°C
2. Neuronal maintenance medium (N2): 1X MEM (Invitrogen), 0.6% D-glucose, 0.1% ovalbumin, 1 mM sodium pyruvate, 5 μg/ml insulin, 20 nM progesterone, 100 μM putrescine, 30 nM selenium dioxide, 100 μg/ml apo-transferrin. Filter-sterilize and store at 4°C. Keep component stock solutions at -20°C
3. Glial and neuronal plating medium (MEM 10): Minimum essential medium (1X MEM, Invitrogen), 10% heat inactivated horse serum (Invitrogen), 0.6% D-glucose, 1 U/100 μg penicillin/streptomycin. Store solution at 4°C
4. Plating Medium: MEM10 containing Minimum Essential Medium (Invitrogen), 10% heat-inactivated horse serum, 0.6% glucose, and penicillin (1 U)/streptomycin (100 μg).
5. Maintenance Medium: N2 medium, containing MEM, 0.6% d-glucose, 0.1% ovalbumin, 1 mM sodium pyruvate, 5 μg/mL insulin, 20 nM progesterone, 100 μM putrescine, 30 nM selenium dioxide, 100 μg/mL apo-transferrin

Abbreviations

1. E18: Embryonic day 18
2. CMF-HBSS: Calcium and magnesium-free Hank's balanced salt solution
3. MEM 10: Glial and neuronal plating medium
4. N2: Neuronal maintenance medium
5. BSS: Balanced Salt Solution

Protocol

1. Sacrifice an embryonic day 18 (E18) pregnant Sprague–Dawley rat.
2. Working in a horizontal laminar hood, remove the fetuses from the uteri, and remove their brains.
3. Place the brains in a sterile culture dish containing 5 mL of calcium- and magnesium-free 1x Hanks balanced salt solution, supplemented with 9.9 mM HEPES, and 1 U/100 µg of penicillin (1 U)/streptomycin (100 µg) in water (BSS).
4. Under a dissecting microscope, remove the meninges of each hemisphere and dissect the hippocampi.
5. Place the hippocampi in a sterile 60 mm dish containing 3–4 mL BSS.
6. Transfer the hippocampi to a sterile conical tube containing 5 mL 0.25% trypsin in BSS.
7. Incubate in a water bath at 37°C for 15 min. Gently remove the trypsin solution and add 5 mL of BSS and let stand for 5 min at room temperature.
8. Repeat twice more and finally add 0.5 mL of BSS per embryo dissected.
9. Using a Pasteur pipette followed by a fire-polished pipette (the tip diameter of which should be half of the regular diameter), gently dissociate the cells.
10. Determine the density of the cells using a hemocytometer.
11. Centrifuge dissociated hippocampal neuronal suspension (2 x 10^6 cells) at 500 x *g* for 6 min.
12. Remove supernatant completely without disturbing the soft neuronal pellet.
13. Resuspend the neurons in the premixed nucleofection solution (82 µL of nucleofection solution and 18 µL of supplement solution, Lonza, Allendale, NJ). Avoid the formation of air bubbles.
14. Remove the 100 µL of cell suspension and mix with cDNA (1–5 µg cDNA in up to 15 µL of deionized water or Tris/HCl 1 mM EDTA 1 mM, pH 8.0. DNA purity should be measured by the ratio of absorbance (*A*) at 260–280 nm. The A_{260}:A_{280}ratio should be at or above 1.8).
15. Add the mixture to the cuvette avoiding the formation of air bubbles.
16. Place the cuvette in the Nucleofector™ and start the machine using the program O-03.
17. Remove the suspension using a transfer pipette and dilute the cells in warmed plating medium (MEM10) containing Minimum Essential Medium (Invitrogen), 10% heat-inactivated horse serum, 0.6% glucose, and penicillin (1 U)/streptomycin (100 µg).
18. Plate neurons on Carolina™ Assistant-Brant Cover glasses (12 mm).
19. Four hours after plating, place coverslips cell-side-down into dishes containing cultured astrocytes.
20. Keep in maintenance medium (N2 medium, containing MEM, 0.6% d-glucose, 0.1% ovalbumin, 1 mM sodium pyruvate, 5 µg/mL insulin, 20 nM progesterone, 100 µM putrescine, 30 nM selenium dioxide, 100 µg/mL apo-transferrin) until processed for cell viability assays.

Notes:

- The experimental protocol should be approved by the Institution Animal Care and Use Committee in accordance with United States Public Health Service regulations and

applicable deferral and local laws before performing any experiments using vertebrate animals

- Embryonic day 16 (E16) pregnant mice could also be used
- Nucleofection solution should be used at room temperature
- Do not exceed 150 µL of final volume in the nucleofection cuvette
- Dilute the nucleofected neurons immediately in warm culture medium
- As positive control, use neurons nucleofected using a GFP-plasmid