

AMAXA Nucleofection Protocol for Rat Cortical/Hippocampal Neurons

Preparation:

DMEM/CS in (1) 24 well plate and (2) 10ml in 10cm plate

DNA at RT

Nucleofection solution at RT

- To do/prepare before completing neuronal prep:
 1. If you are using self-prepped DNA, it MUST be very high quality/purity for AMAXA → Use Qiagen Endotoxin-Free Kit and re-suspend @ 1-5 μ g/ μ L in Endotoxin-Free TE Buffer (can also Phenol:Chloroform extract, EtOH precipitate to further increase purity) before using DNA for Nucleofection.
 2. Make up DMEM (4.5g/L glucose) + 10% Supplemented Calf Serum (CS+).
 3. Pre-incubate DMEM/CS+ in desired plates @37°C for 1 hour to equilibrate.
 4. Pre-incubate 10mL DMEM/CS in a Petri dish @37°C for 1 hour to equilibrate.
 5. Warm Nucleofection Solution (w/added supplement) to room temperature.
 6. Aliquot DNA samples into Eppendorf tubes, at room temperature. Want 1-3 μ g total DNA in no more than 5 μ L (AMAXA pmax-GFP positive control: 4 μ L).
 7. Fire-polish a glass Pasteur pipette to obtain a smaller bore size (\leq 1/8 original).
 8. Set-up AMAXA device (get from Carmen in GIVI) with desired Program (suggest Q-003, Q-004, or Q-005 for these neuron types), and place it in the tissue culture hood.
- After neuronal prep and counting:
 9. NOTE: Do not dilute cells to plating volume at this step - keep at [high].
 10. Use a P1000 pipette to aliquot ~5-6x10⁶ cells/Eppendorf tube (one tube/transfection condition).
 11. Spin in tabletop centrifuge @ ~90xg for 8 minutes, with “soft” spin.
 12. QUICKLY aspirate off OptiMEM+Glucose with the fire-polished glass pipette, or by using a P200 pipette tip on the end of the glass pipette to reduce the bore. (A P200 pipettelman may also be used to remove the last bit without disturbing the pellet.)
 13. NOTE: Remove as much media as possible without disturbing the delicate pellet.
 14. Immediately add 100 μ L of Nucleofection Solution, and re-suspend neurons.
 15. NOTE: DO NOT re-suspend by pipette → MUST FLICK-MIX thoroughly. Nucleofection solution is toxic, and membrane shearing caused by mechanical pipetting will cause excessive cell death. Also, DO NOT keep neurons in this solution for more than 15 minutes (may have to stagger Steps 11-22 if you have more than 2-3 conditions).
 16. Using P200, carefully aspirate 100 μ L of re-suspended cells and add to DNA in DNA tube. Gently mix by flicking, and avoid making bubbles.
 17. Then carefully transfer cell/DNA mix to bottom of cuvette by placing P200 pipette tip along side of cuvette. Again, avoid making bubbles.
 18. Put on blue cap, and gently flick cuvette to eliminate any bubbles.
 19. Take Petri dish with equilibrated DMEM/CS out of incubator.
 20. Place cuvette into AMAXA machine, and press “X” button to initiate electroporation (takes ~10 seconds).
 21. When it says “OK”, QUICKLY add 500 μ L (using P1000) of still-warm DMEM/CS to cuvette, then use AMAXA-certified plastic bulb pipette to carefully and slowly transfer to a clean Eppendorf tube. Repeat addition of fresh 500 μ L DMEM/CS to cuvette and transfer to same Eppendorf. Then incubate Eppendorf tube @37°C for ~10-30 minutes.
 22. After this incubation, gently re-suspend any settled cells with a P1000, then seed cells into equilibrated DMEM/CS+ plates, and incubate for 1-2 hours. RECOMMENDED: Recount neurons after re-suspension to ensure plating at a uniformly high density → Survival of nucleofected neurons increases significantly at a higher cell density, increasing overall transfection efficiency.

23. Pre-warm standard Neuronal Culture Media + Serum @37°C (water bath).
 24. After a 1-2 hour incubation check that the neurons have settled, then gently aspirate DMEM/CS+ and replace with the normal volume of standard Neuronal Culture Medium + Serum.
- Vendors/Catalogue #'s:
 - Qiagen EndoFree Maxi Plasmid Kit (#12362)
 - AMAXA Rat Cortical/Hippocampal Neuron Nucleofactor Kit (#VPG-1003)
 - Invitrogen/Gibco RPMI Media 1640, 1L (#22400-071)
 - Invitrogen/Gibco DMEM with High Glucose, 500mL (#11960-044)
 - Hyclone Supplemented Calf Serum, 500mL (#SH30072.03)