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Protocol for magnetofection of neurons and immunofluorescence staining

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Magnetofection

1. Work in the laminar flow hood with sterile solutions
2. Transfer 1 mL of culture medium from each coverslip-containing wells into a fresh 12-well plate and place in the incubator.
3. Prepare transfection mix (amount per coverslip):

    Tube A: 30 μL OPTI-MEM medium + appropriate DNAs (total 1 μg)

    Tube B: 30 μL OPTI-MEM medium+ 2 μL Lipofectamine 2000; incubate 5 min at RT

    Dilute magnetoFECTION reagent 1:10 in OPTI-MEM medium. Vortex the diluted mix shortly

4. Mix Tube A and Tube B; add 2 μL diluted MagnetoFECTION to the combined mixture and incubate 15 min at RT
5. Add the complete mixture to coverslips in 1 mL medium, with cells facing upwards (incubate at 37º C/5% CO_2 for 25-45 min)
6. Flame the forceps and remove the coverslips from the transfection medium and transfer to the 12-well plate containing 1 mL conditioned medium (1 mL removed at the beginning).

Immunofluorescence staining of transfected neurons

Check the cells under the microscope to make sure they look healthy and are alive, place them back in the incubator.

Prepare the following before removing the cells out of the incubator to stain:

1. Thaw 4% PFA solution (1 mL per coverslip), bring it to RT, and distribute it into a 12-well plate
2. Prepare 0.1% Triton X100 + PBS + 10% NGS (1 mL per coverslip), and distribute it into a second 12-well plate
3. Pipet PBS into a third 12-well plate to rinse the coverslips
4. Prepare the primary antibody mixtures with appropriate dilution in PBS+ 10% NGS

Staining:

1. Remove the coverslips from culture medium and rinse them by dipping gently in PBS and immediately transfer them into the fixative (incubate 10-15 min at RT)

2. In the mean time, fold tissue paper into 4 and place on a large glass/ plastic plate and wet the tissue to maintain humidity and prevent antibody evaporation
3. Cut parafilm to required size, mark with felt pen the required coverslips, and place it on top of wet paper
4. After fixation, rinse the coverslips in PBS again to remove the fixative and move them into wells containing the Triton X100 solution; incubate 3-5 min max
5. Pipet 120 μL antibody mix per coverslip onto the labeled parafilm; transfer the coverslips up-side-down onto the antibody drop and incubate 60-90 min at RT
6. Wash 3 x 5 min in PBS
7. Prepare secondary antibody solution with appropriate fluorophores (1:500 dilution in PBS with 10% NGS for all Alexa 488, Cy3 and Cy5)
8. Place a fresh parafilm with correct labels on top of the wet tissue like before, and pipet 120 μL of the secondary antibody mix per coverslip
9. Transfer the coverslips onto the antibody mix and incubate 30 min at RT
10. Wash 3 x 5 min in PBS
11. Air dry the coverslips on fresh tissue and place inverted on gelatin-coated glass slides with correct labeling (date, culture age, tranfected plasmids, staining)
12. Dako mounting medium to be used only when storing coverslips for long periods (1-3 months) otherwise use glycerol mounting medium (1:2 in sodium acetate buffer, pH 9.5).