Calcium Phosphate Transfection Method

Reference

1. Kwon, M., & Firestein, B. L. (2013). DNA transfection: calcium phosphate method. *Methods in molecular biology (Clifton, N.J.)*, *1018*, 107–110. https://doi.org/10.1007/978-1-62703-444-9_10

Solutions

1. Transfection medium: Neurobasal medium with 2% B27 supplement
2. 2x HEPES-buffered saline (2x HeBS): 274 mM NaCl, 10 mM KCl, 1.4 mM $Na_2HPO_4 \cdot 7H_2O$, 15 mM dextrose, 42 mM HEPES in 180 ml H_2O and adjust pH to 7.03 with 5 N NaOH. Bring to 200 ml with H_2O. Adjust pH to 7.12 with 1 N NaOH. Sterile filter and aliquot 1 ml in microcentrifuge tubes. Store up to several month at −80°C

Abbreviations

1. HeBS: HEPES-buffered saline

Protocol

1. Equilibrate transfection medium in 37°C, 5% CO_2 incubator overnight.
2. Thaw and aliquot 15 µl of 2x HeBS per well (of a 24 well plate) in a microcentrifuge tube.
3. Prepare $CaCl_2$–DNA precipitates in microcentrifuge tubes.
    a. 1.5 µl of 2.5 M $CaCl_2$.
    b. 3 µg of DNA.
    c. Add H_2O up to 15 µl.
4. Add $CaCl_2$–DNA dropwise to the tube of 2x HeBS while swirling.
5. Incubate in the dark for 25 minutes at room temperature.
6. While incubating, prepare cells.
    d. Collect/save the medium from the well into 50 ml conical tubes.
    e. Wash cells with transfection medium four times.
    f. Add transfection medium into the well and put in incubator.
7. Add 30 µl of transfection mixture dropwise over the culture.
8. Shake the plate or rock back and forth gently and incubate for 40 minutes.
9. Aspirate the transfection medium and wash two times with fresh transfection medium.
10. Replace with conditioned medium (saved from step 6).