

Calcium phosphate transfection (Elena, April, 2005)

Protocol for 35 mm Petri dishes

- plate the cells the day of transfection (or the evening before) to a confluence of 40-50%; alternatively prepare as many dishes as needed for experiments with few and isolated cells
 - change the medium (pre-warmed at 37 °C) 1-2 h before transfection (1.5 ml each dish)
 - pre-warm solutions for transfection at room temperature
 - to prepare the calcium-phosphate-DNA co-precipitate prepare in two 1.5 ml eppendorfs the following solution:
 - sol. A : 4 µg of plasmid DNA, 7.6 µl of CaCl₂ 2 M and bring to a final volume of 62 µl with Tris-HCl 2 mM (pH 7.6).
 - sol. B : 62 µl of HBS
- Add solution A drop by drop (1 drop/sec) in solution B at room temperature. Tap the side of the tube and incubate at room temperature for 20-30 min.
- Transfer the calcium-phosphate-DNA suspension into the Petri dish. Add the suspension drop by drop (1 drop/sec) and gently mix by moving the dish.
 - incubate the cells at 37 °C for 4-16 h in a humidified incubator.
 - wash the cells with PBS and add pre-warmed complete growth medium.

Solutions:

HBS : 50 mM Hepes
1.5 mM Na₂HPO₄
280 mM NaCl
pH 7.13 (should be precise) with NaOH

TRIS-HCl : 2 mM, pH 7.6

CaCl₂ : 2 M

The following conditions should be checked carefully and be optimized:

- density and growth conditions of cells
- pH of the HBS buffer
- amount of plasmid DNA
- purity of plasmid preparation