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## Calcium-Phosphate Transfection (optimised for 293 cells)

### Buffers

- HeBS-Buffer (Hepes buffered saline)

400 ml A.dest.	final conc.
8 g NaCl	280 mM
0.2 g Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O (or 0.107 g anhydrous)	1.5 mM
6.5 g Hepes (Sigma H-7006) (or 5.96 g of free acid)	50 mM

Adjust the pH to exactly 7.05 (calibrate pH-meter with pH 4.01 and pH 7.00 buffers before).

Add A.dest. to 500 ml, filter through 0.2 µm filters and store in aliquots at -20°C (not longer than 6 months). Thawed aliquots shouldn't be frozen again.

- CaCl<sub>2</sub>: 29.4 g CaCl<sub>2</sub>·2H<sub>2</sub>O (MW=147) in 100 ml A.dest (final conc.: 2 M)

Filter through 0.2 µm filters and store aliquoted at -20°C.

- Chloroquine (optional): chloroquine·2H<sub>2</sub>O (Sigma C-6628): 12.9 mg/ml in PBS (conc.: 25 mM). Filter through 0.2 µm filters and store at -20°C.

### Procedure (amounts are given for 6-wells):

1. Seed cells (about 500 000 cells per 6-well = per 10 cm<sup>2</sup>) one day before the transfection (in DMEM/10% FCS)
2. (Optional: 1 h before transfection, exchange the medium for medium containing 25µM chloroquine)
3. Thaw HeBS and CaCl<sub>2</sub> at room temperature
4. For each transfection prepare aliquots of 71 µl HeBS
5. Prepare the DNA/ CaCl<sub>2</sub>-Mix: 4 µg DNA (total) in 62 µl A.dest. + 9 µl CaCl<sub>2</sub>
6. Add the DNA/ CaCl<sub>2</sub>-Mix drop-wise to the HeBS aliquots (by screwing the Gilson pipette) and slightly mix after each drop. Incubate for 2 - 3 min at R.T. to form the DNA-precipitate (not longer).
7. Add the DNA-precipitate drop-wise to the cells (by screwing the Gilson pipette and moving it to cover the whole surface of the cell culture; don't swirl the dish).
8. Carefully transfer the dish back to the incubator. Incubate for 24 h (or in the presence of chloroquine: for 10 h) and exchange the medium afterwards. (The transfection is in the presence of FCS!). The efficiency of transfection is in

the range of 70-90%. Harvest the cells after 48 h.

The protocol is adapted from Neil Perkins who adapted it from Gary Nolan in 1995

(See web site: <http://www.stanford.edu/group/nolan/>

or CP in Mol.Biol. 9.1 and 9.11.2-3)