

Calcium Phosphate Transfection

Works very well for 293 cells (up to 95% transfection efficiencies). Grow cells in standard DMEM + 10% FCS + Pen/Strep at 37°C and 5% CO₂ and split 1/10 every few days so they do not become overconfluent.

1. Split cells to about 20-30% confluency at least 2 hrs. prior to transfection. This gives them enough time to settle back down.
2. Thaw 2X HBS at room temperature (37°C is NOT recommended). Do not re-freeze HBS after use, just discard (for this reason, aliquot original stock into suitable volumes).
3. Prepare CaCl₂/HBS/DNA precipitate:

For each 10 cm dish, aliquot 0.5 ml 2X HBS into a sterile 1.5 ml microfuge tube (*0.18 ml for a 6 cm dish*). In a separate tube, aliquot 10 µg of DNA, 61 µl of 2M CaCl₂ and enough distilled water to bring the total volume to 0.5 ml (*5 µg, 22 µl and 0.36 ml total volume for a 6 cm dish*). Add the CaCl₂/DNA mix to the HBS slowly with a P1000 pipette, mixing gently during the addition. Add directly to cells by dropping slowly and evenly into medium, trying to cover as much of the plate as possible. Do not mix, but simply carry the dish to the incubator (this mixes it) and place at 37°C/5% CO₂ for 16-24 hrs. Remove media and wash cells once with warm PBS, then add fresh, warm complete medium and resume incubation for 24-60 hrs. prior to assaying.

2X HBS

8g NaCl

0.2 g Na₂HPO₄-7H₂O (0.14 g if using dihydrate; [phosphate] must be 1.5 mM)

6.5 g HEPES

pH to 7.0 and bring up to 500 ml with distilled water. Re-pH at this point because it can change. Aliquot and store at -20°C.

2M CaCl₂

87.6 g CaCl₂-6H₂O in a final volume of 200 ml distilled water. Filter sterilize and store at 4°C (DO NOT FREEZE).

