

Version 2

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Calcium phosphate transfection mammalian cells V.2

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Works for me

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ABSTRACT

This is our calcium phosphate transfection protocol. It always works with HEK293T cells, for other lines the transfection efficiency depends on their source: some HeLa and U2OS clones can be transfected using CaPO₄, however most cannot. We mostly use this method to transfect HEK293T cells for lentivirus production.

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MATERIALS TEXT

10x HBS-buffer:
40 gr. NaCl
25 gr. Hepes
1.85 gr. KCl
0.63 gr. Na₂HPO₄·H₂O
500ml ddH₂O

Store in aliquots of 50mL at -20°C.

1x HBS-buffer:

Dilute 50mL 10x HBS-buffer in a total volume of 450mL ddH₂O and adjust pH to **6.95** with NaOH. Adjust the volume to 500mL with ddH₂O.

Sterilize by passage through a 22mm-filter. Store in 50 mL aliquots at 4°C.

2.5M CaCl₂:

18.4 gr. CaCl₂·2H₂O in 50 mL ddH₂O.
Sterilize by passage through a 22mm-filter. Store at 4°C.

10x HEPES

5 gr. KCl
83 gr. NaCl
24 gr. HEPES
- or - 26 gr. Na-HEPES

Add ±800 mL ddH₂O.
Adjust the pH to **7.3**, adjust volume to 1L and autoclave.

1x HEPES

Dilute 50mL in 450mL sterile ddH₂O to make 1x HEPES. Or dilute in 450 mL ddH₂O and autoclave.

DISCLAIMER:

This protocol is a mix of the protocols I've been taught during my master internship in René Bernards lab and during my PhD in Bernhard Lüscher's lab.

BEFORE STARTING

The **CRUCIAL** part in this protocol is the adjustment of the pH of the HBS buffer **EXACTLY**. We do this at room temperature with room temperature water.

Day 1 1d

- 1 Seed cells: density depends on cell line and dish used. For example in a 10cm dish seed 0.8×10^6 HeLa or 1.5×10^6 HEK293T cells; in a 6-well plate seed 0.3×10^6 HEK293T cells or 0.15×10^6 HeLa cells.

Day 2 7h

- 2 

Observe the cells under the microscope.

Prepare the transfection mix. The amounts given here are for a 10cm dish and can be adjusted to other dishes and plates.

Prepare in an eppendorf tube:

20 µg plasmid DNA

Add  950 µl HEBS buffer

Add  50 µl CaCl₂

We rarely use a full 20µg of DNA encoding for something: some times as little as 2 µg of the total is encoding for a protein of interest, whereas the rest is an empty vector. This amount of DNA is required to efficiently form precipitates.

3




20m

Flick the tube gently to thoroughly mix and incubate at  **Room temperature** for approximately  **00:20:00**.

4



6h

Add the mixture drop-wise onto the cells, gently agitate the dish to evenly distribute the mixture and place the cells back in the incubator for 5-6 hours ( **06:00:00**).

5

After 5-6 hours, remove the media, add warm HEPES buffer and incubate 5-10 minutes ( **00:10:00**).

10m

6

Remove the HEPES and add fresh full DMEM. The precipitates are visible under a microscope and should disappear during the incubation with HEPES. Replace the cells in the incubator.

Day 3

7

Day 3-4

Process your cells 24 to 48 hours after transfection, incubation time depends on experimental needs and set-up. If it does not interfere with the experiment, 5% of the transfected plasmid DNA can be a GFP-encoding plasmid to monitor transfection efficiency.