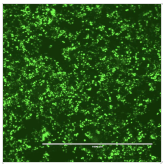

Version 1

Mar 19, 2021

Calcium phosphate transfection mammalian cells V.1

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1 Works for me dx.doi.org/10.17504/protocols.io.bthnnj5e

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SUBMIT TO PLOS ONE

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 Lüschers lab.

BEFORE STARTING

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

1 Day 1

Seed cells: density depends on cell line and dish used. For example in a 10cm dish seed 0.8x10^6 HeLa or 1.5 x10^6 HEK293T cells; in a 6-well plate seed 0.3 x10^6 HEK293T cells or 0.15x10^6 HeLa cells.

2 Day 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:
20µg plasmid DNA
Add 950µl HEBS buffer

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Add 50µl CaCl2

Flick the tube gently to thoroughly mix and incubate at room temperature for approximately 20 minutes.

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 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.

4 After 5-6 hours, remove the media, add warm HEPES buffer and incubate 5-10 minutes. 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.

5 **Day 3-4**

Process your cells 24 to 48 hours after transfection, incubation time depending 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.

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