

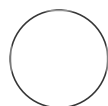


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# Lentivirus production in HEK293FT with calcium-phosphate method

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## ABSTRACT

To produce lentivirus, HEK293FT cells are transfected with lentiviral vector and packaging plasmids using the calcium-phosphate method.

## OPEN ACCESS

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**Protocol status:** Working  
We use this protocol and it's working

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## Day1

- 1 Seed 6 million HEK293FT in a 10cm plate with 10 ml complete medium (DMEM+10%FBS+1% pen-strep+1% MEM-NEAA)

## Day 2

- 2 Add 25  $\mu\text{l}$  from 10 mM Chloroquine (Final Concentration:25 $\mu\text{M}$ ) to 9 ml of complete medium. (To avoid lysosomal degradation of the transfected DNA)
- 3 While preparing the plasmid mix, keep the plates inside the incubator.
- 4 In 2ml tubes, prepare the transfection mixture in the following order:

Ingredients	Stock solution Conc. / mass
pCMV-VSV-G	4 $\mu\text{g}$
psPAX2	7.5 $\mu\text{g}$
Lentiviral Vector	10 $\mu\text{g}$
ddH <sub>2</sub> O	variable
Total value: 437.5 $\mu\text{l}$	
2M CaCl <sub>2</sub> (ice-cold)	62.5 $\mu\text{l}$

Transfection mix for Lentivirus production.

- 5 500  $\mu\text{l}$  of ice-cold HBS-HEPES buffer saline is added to the mix in a slow dropwise manner.
- 6 The Solution was further mixed by passing the tube on a tube rack for 30-45 seconds. until the mixture is full of bubbles.
- 7 After 5-7 minutes at RT the mixture was added to the plate drop by drop and with a circular motion to cover the surface of the plates.

8 After 6 hours, the medium was changed.

## Day 4

9 The medium was collected 48 hours post-transfection and filtered through 0.45 µm filter

10 The lentivirus-containing medium was aliquoted and stored at -80°C for later use.