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

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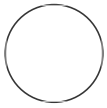
Lentivirus Production

Yiqin Shen[1]

[1]University of California, San Diego



Yiqin Shen
University of California, San Diego

ABSTRACT

A protocol to produce lentivirus through cell transfection

Day 1

1 Coat 15ml cell culture plates with D-poly-lysine (50ug/ml) for 00:30:00 to 01:00:00 .
Wash plates with cell culture water for 1 to 3 times. Let the plates to air dry.

2 Plate pre-grown low passage HEK cells at 6e^6 cells per plate.
Add 20ml of media.
Incubate overnight at 37 °C 5% CO2.

> **Note**
>
> Cell culture media: DMEM/F-12, GlutaMAX™ supplement + 10% FBS + 1% P/S

Day 2

3 Change media about 3 hours prior to transfection.

4 Transfect cells with viral components using CaCl2 transfection Kit in the afternoon.

4.1 Each plate = 20/25ug desired plasmid, 10.2ul PMD26, 15.6ul PsPAX, 93 ul CaCl2; add water to 750 ul; 750 ul HBSS

4.2 Add HBSS to tube 2; add plasmid, CaCl2, H2O to tube 1, and mix by vortex.

4.3 Add tube 1 to tube 2 (on medium speed vortex) drop by drop (to form droplets).

4.4 Wait 00:30:00 ; vortex again before use. 30m

Add 1.5 mL to each plate, distribute evenly across the plate.
Rock the plates gently to allow more distribution.

5 Incubate overnight at 37 °C 5% CO2.

Day 3

6 Change media for all of the plates. Add 20 mL fresh media.

Day 4

7 Collect media from the plates (~24 hours after media change). Store at 4 degrees.

8 Add 20 mL of fresh media.

Day 5 & 6

5h 25m

9 Repeat collection as day 4.

10 Spin down collected media at 500 x g, 4°C, 00:10:00 . 10m

11 Add 36ml of supernatant from step 10 to 12 ml of Lenti-X concentrator. Incubate the mixture at 4 °C in a rocker, for 04:00:00 to Overnight . 4h 30m

12 Centrifuge the mixture from step 11 at 1500 x g, 4°C, 00:45:00 . After centrifugation, an off-whit 45m pellet will be visible.

13 Remove supernatant from the tubes.
Resuspend the pellet in 100ul HBSS per pellet.
Aliquote 200ul per freezing tube.
Place the tubes on ice or freezing blocks immediately when you are finished, and transfer to -80 °C for long term storage.