

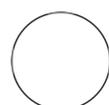


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Lentivirus production

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The AAA+ chaperone VCP disaggregates Tau fibrils and generates aggregate seeds
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ABSTRACT

This protocol describes the production of lentiviruses to transduce HEK293T cells and has to be performed in a biosafety level 2 laboratory

SAFETY WARNINGS

Has to be performed in a biosafety level 2 laboratory.

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

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Lentivirus production

1d 1h 10m

- 1 Plate $\sim 3.6 \times 10^6$ Lenti-X HEK293T cells (Takara) in 10 cm dish in 10 mL standard DMEM. Cells should be $\sim 80\%$ confluent at the time of transfection.

Note

NOTE: Only low passage cells should be used.
- 2 Next day, remove 5 mL medium and replenish with fresh medium.
- 3 Warm up reduced serum medium e.g. Opti-MEM (Gibco) and transfection reagent to room temperature (RT). This protocol was performed with Lipofectamine 3000 transfection reagent (Thermo).
- 4 Add 24 μL Lipofectamine 3000 to 600 μL Opti-MEM, mix by vortexing and incubate 5 min at RT.

5m

- 5 In another tube, mix 6 µg plasmid containing gene of interest, 5 µg packaging plasmid psPAX2 (RRID:Addgene_12260), 1 µg envelope plasmid pMD2.G (RRID:Addgene_12259) and 24 µL P3000 reagent (provided by manufacturer along with Lipofectamine 3000 reagent) in 600 µL Opti-MEM, mix by vortexing and incubate 5 min at RT.
- 6 Mix contents of both tubes and incubate for 15 min at RT. 15m
- 7 Add DNA-lipid complex to cells dropwise.
- 8 2 days later, collect virus-containing medium and centrifuge for 5 min at 1,000 x g. 2d
- 9 Collect supernatant in a fresh tube and proceed with concentration.

Concentration

1d 1h 10m

- 10 Add Lenti-X concentrator (Takara) to clarified virus-containing medium at 1:4 dilution and mix well by gently inverting tube.
- 11 Incubate overnight or 2 h at 4 °C. 1d
- 12 Next day, centrifuge for 45 min at 1,500 x g at 4 °C followed by gently aspirating supernatant. 45m



13 Resuspend viral pellet in 100 - 1000 μ L PBS, aliquot and store at -80 °C until use.