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Spinfection for Suspension and Adherent Cells

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol is for infection of adherent cells (such as HEK293t) with prepackaged lentivirus. Different cell lines will have different infection rates, and the infectivity of lentivirus stocks will also depend and vary. Optimization will be required to obtain infection, as well as to determine the appropriate proportion of cells and virus to obtain the desired MOI>

Guidelines

When using lentivirus, adhere to the proper safety measures and precautions. Proper care should be taken that all waste is disposed in biohazard containers or bleached. Double gloves are recommended.

Materials

Adherent Cells and appropriate media


Lentivirus

Polybrene

 Polybrene Infection / Transfection Reagent **Merck Millipore (EMD Millipore) Catalog #TR-1003-G**

Troubleshooting

Safety warnings

 When using lentivirus, adhere to the proper safety measures and precautions. Proper care should be taken that all waste is disposed in biohazard containers or bleached. Double gloves are recommended.

Before start

Make and prepackage your virus.




Plating cells

- 1 For HEK293t cells, plate 50,000 cells/well in 0.5 mL of DMEM on 24-well plate the day prior.

30m

Spinfection

2h 30m

- 2 30+ min before spinfection, make sure the table-top centrifuge is at 30 °C .
- 3 Add lentivirus and polybrene to each well.
- 3.1 Virus amount
Virus titer will vary, so it is useful to add different amounts of virus to well to determine the appropriate titer. For a well of a 24-well plate, virus amounts can range from 5 µL to 500 µL.
- 3.2 Polybrene amount
For most cell types polybrene increases infection efficiency and should be used at a final concentration of 8 µg/mL. Example dilution: add 0.8 uL (160 µg) polybrene stock (stock at 100mg/mL) to 0.5 mL media.
- 4 Spin at 1000 rcf for 2 hrs at 30 C.
 1000 rcf, 30°C
- 4.1 It is OK if the temperature starts off between 20-30C.
- 5 Remove plate from centrifuge and place in hood. Douse with ethanol and wait 10 minutes. Place in incubator.
- 6 Infection will be achieved within 2-3 days.

2h