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Fluorescence Titering Assay for Lentivirus

Addgene the Nonprofit Plasmid Repository[1]

[1]Addgene

1 Works for me dx.doi.org/10.17504/protocols.io.4xrgxm6

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ABSTRACT

This protocol is for running a fluorescence titering assay for lentivirus. To see the full abstract and additional resources, visit the Addgene protocol page.

Sample Data

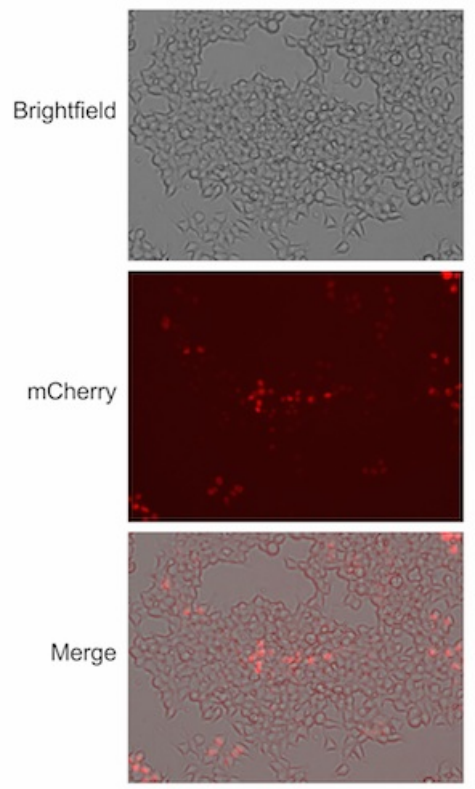

Figure 1: Lenti-X 293T cells were transduced with a range of dilutions of 64108 pHAGE-TO-dCas9-3XmCherry. 72 hours post transduction, cells were assayed for mCherry expression using fluorescence microscopy.

EXTERNAL LINK

https://www.addgene.org/protocols/fluorescence-titering-assay/

GUIDELINES

Workflow Timeline

Day 0: Seed Lenti-X 293T cells (this cell line is optimized for production of lentiviral vectors)

Day 1: Transduce Cells

Day 2 (am): Remove media, replace with fresh media

Day 4-5: Count Fluorescent Cells

MATERIALS TEXT

Reagents

- DMEM high glucose
- L-alanyl-L-glutamine (or alternative stable glutamine)
- Heat-inactivated FBS
- Polybrene
- PBS pH 7.4 without calcium or magnesium (cations can affect the attachment of adherent cells)
- Microcentrifuge tubes
- 6-well dishes
- Pipettes
- Pipette tips
- Lentivirus Preparation

Equipment

- Biosafety cabinet
- Pipetman
- Pipettors
- Incubator
- Fluorescence microscope

Reagent Preparation

DMEM Complete: 10% v/v FBS and 4 mM L-alanyl-L-glutamine

- To a **500 ml** bottle of DMEM high glucose, add **55 ml** of heat inactivated FBS and **11 ml** of 200 mM L-alanyl-L-glutamine. Store at **4 °C** .

Pro-Tip
Different brands and lots of FBS can promote or inhibit transfection. Test a variety of brands and lots of FBS to find one suitable with your protocols. FBS can be purchased already head inactivated or it can be inactivated in the lab by heating to **56 °C** for **00:30:00** .

SAFETY WARNINGS

See SDS (Safety Data Sheet) for safety warnings and hazards.

BEFORE STARTING

Considerations Before You Start

- The health of the the target cell line is critical for obtaining accurate titers.
  - Check the cells for mycoplasma regularly
  - Do not over or under-grow your cells.

- Thaw a new vial of cells after 20-30 passages.

- Do not add penicillin/streptomycin to the media.
- Titer will vary between cell lines.
- It is not recommended that lentiviral supernatants be subjected to multiple freeze-thaw cycles.

Seeding cells

1 Seed 75,000 cells into each well of a 6-well dish.

1.1 Dilute 525,000 cells into **14 ml** of DMEM complete.

1.2 Mix well by pipetting or inverting.

1.3 Aliquot **2 ml** of cell suspension into each well of the 6-well dish.

2 Incubate the cells overnight.

Viral Titering

3 If using freshly collected virus, filter through a 0.45 µm polyethersulfone filter to remove cells and debris.

Lentiviral titer can decrease during cycles of freeze-thaw. If you are freezing and aliquoting virus, it is recommended that you titer from the frozen stock to account for any loss in titer associated with freeze-thaw.

4 If using frozen virus, rapidly thaw the lentiviral aliquot at **37 °C** by agitating in a warm water or bead bath.

5 Prepare dilutions of the lentivirus into DMEM complete containing 10 µg/mL polybrene.

Note, this protocol was developed using low titer lentiviral vectors (10^5TU/mL). If you anticipate that your viral stock will be higher titer consider additional dilutions.

Dilution	Volume of Lentivirus Stock (µL)	Volume of DMEM complete (µL)	Volume of 10mg/mL polybrene (µL)
1:10	150	1348.5	1.5
1:25	60	1438.5	1.5
1:50	30	1468.5	1.5
1:75	20	1478.5	1.5
1:100	15	1483.5	1.5

Mix the dilutions well

6 Gently aspirate media from the cells.

7 Add **1.5 ml** of a viral dilution to each well (each well gets one dilution with one well left over).

8 Count the cells in the remaining well, a cell count is required for calculating the titer.

9 Incubate for **48:00:00** - **72:00:00** .

10 Gently aspirate media and replace with **1 ml** of PBS.

Analysis

11 Calculate the percent of fluorescent-positive cells in each well.

When calculating titer, only consider wells with less than 40% fluorescent-positive cells. Titering methods assume 1 integration event per cell. When the percentage exceeds 40% you risk counting cells with multiple integration events leading to underestimation of the true titer.

12 Calculate the transduction units per mL (TU/mL) using either the dilution factors (method 1) or the volume of virus (method 2):

12.1 Method 1: TU/mL = (Number of cells transduced x Percent fluorescent x Dilution Factor)/(Transduction Volume in mL)

Method 1 example: If the 1:100 well has 25% fluorescent cells and 150,000 cells were originally transduced, then there are
(150,000 x 0.25 x 100)/(1.5 mL) = $2.5x10^6$TU/mL

12.2 Method 2: TU/mL = (Number of cells transduced x Percent fluorescent)/(Virus volume in mL)

Method 2 example: If 15 µL of virus added to 150,000 cells resulted in 25% fluorescent cells, then there are
(150,000 x 0.25)/(0.015 mL) = $2.5x10^6$TU/mL

13 For a more accurate titer, take the average of multiple dilutions.