



Lentiviral Titer by Limiting Dilution

Materials

Solutions

Hexadimethrine bromide (2 mg/mL stock) Product No. [H9268](#)

Puromycin (10 mg/mL stock) Product No. [P9620](#)

Crystal Violet Solution, Product No. [HT90132](#)

Dulbecco's Phosphate Buffered Saline, Product No. [D8662](#)

Lentiviral Particles

Choose one of the following:

MISSION TRC shRNA Lentiviral Particles Target Set

shRNA Non-Target Control (Product No. [SHC002V](#))

Positive Control shRNA Lentiviral Particles

JAK1 Control Lentiviral Particles (Individual construct TRCN0000003102)

Media

Minimum essential medium containing 10% fetal calf serum or optimized growth media specific for cell line

Special Equipment

6-well cell culture treated plates

15 mL conical vials

Cells

Rapidly growing cultures of mammalian cells

Method

Day 1. Add approximately 2.0×10^5 cells in fresh media to all wells of a 6-well plate. Incubate 18-20 hours at 37°C in a humidified incubator in an atmosphere of 5-7% CO₂.

① Growth rate of cells vary greatly. Adjust the number of cells plated to accommodate a confluency of 30-50% upon transduction.

Day 2. Thaw lentiviral particles at room temperature until ice crystals disappear. Mix by gently tapping the tube several times with a finger. Store the lentiviral stock on ice.

⚠️ Lentiviruses are quite labile. Multiple freeze-thaw cycles and prolonged exposure to ambient temperatures will decrease the lentiviral titer.

Prepare 15 mL of media containing hexadimethrine bromide (final concentration 8 µg/mL).

🚫 Hexadimethrine bromide enhances transduction of most cell types. However, some cells, like primary neurons, are sensitive to hexadimethrine bromide. Do not add hexadimethrine bromide to these types of cells. If working with a cell type for the first time, a hexadimethrine control only well should be used to determine cell sensitivity.

Prepare 2 mL ten-fold serial dilutions over a range of $1(10)^{-2}$ to $1(10)^{-6}$ in 15 mL conical vials with the chosen lentiviral construct. Mix gently by inverting the tubes five to ten times.

Add 1 mL of medium containing hexadimethrine bromide to one well of the 6-well plate. Then add 1 mL of each of your lentiviral dilutions to the remaining wells of the plate. Incubate 18-20 hours at 37°C in a humidified incubator in an atmosphere of 5-7% CO₂.

① 1 mL of media/lentiviral dilution is added to the wells on day 1. 2 mL media is used for the remaining incubations. The decreased volume during the initial incubation allows for optimal contact between the virus and cells.

Day 3. Remove the media containing lentiviral particles from wells. Add 2 mL fresh media (without hexadimethrine bromide) to each well.

⚠️ Slowly add the media to the inner wall of the well, allowing it to run down the wall and into the well. Some cell lines, such as HEK293T, are loosely attached to the surface of the plate and slough off easily when agitated.

Day 4. Remove media from wells. Add fresh media containing puromycin.

⚠️ The appropriate concentration of puromycin for each cell type is different. If the concentration for the desired cell type is unknown, a titration experiment must be performed. Typically, 2-10 µg/mL are sufficient to kill most untransduced mammalian cell types.

Days 5 – 14. Replace media containing puromycin as necessary during the selection process (usually every 2 to 4 days).

Day 14. Remove media and gently wash each well with PBS. Add 1 mL of crystal violet solution and incubate 10 minutes at room temperature.

⚠ Slowly add the media to the inner wall of the well, allowing it to run down the wall and into the well.

Remove crystal violet solution. Wash with 3 mL PBS. Repeat.

Count the blue-stained colonies using a microscope at a magnification of 40X.

Determine the lentiviral titer, defined here as Transforming Units per milliliter (TU/mL).

Calculate by multiplying the number of colonies per well by the dilution factor.

Notes:

Puromycin titration (kill curve) should be performed when working with a new cell type.

1. Plate 1.6×10^4 cells into wells of a 96-well plate with 120 μ l fresh media.
2. The next day add 500 – 10,000 ng/mL of puromycin to selected wells.
3. Examine viability every 2 days.
4. Culture for 10 – 14 days. Replace the media containing puromycin every 3 days.
5. The minimum concentration of puromycin that causes complete cell death after 3-5 days should be used for that cell type.