DATA SHEET

Cat. No.	Description	Quantity
G074	Cationic Lipid	1.0ml

Description

Lentifectin™ is a transfection reagent specially formulated with multiple cationic polymers for the production of Lentiviral vectors *in vitro*. It is shown that Lentiviral vectors produced with Lentifectin™ consistently have higher titers than those produced with Calcium-phosphate transfection or with other types of lipid transfection reagents.

Protocol

The following protocol allows the production of recombinant lentiviral vector up to 10IU/ml. We recommend including a negative control (no DNA, no transfection reagents) in your experiment to help you evaluate your results. You will need 1.2×10^7 293FT cells for each sample.

DAY 1:

· Seed 293T cells into 10 cm dishes (add ~$1.2X10^7$ cells per 10 cm dish).

DAY 2:

· Check to make sure the cells are 80-90% confluent;
· For each 10cm dish prepare transfection complex as follows:
a. Solution A: Dilute 25ug DNA plasmids(total) in 1 ml serum-free medium;
b. Solution B: Dilute 100ul LentiFectin™ reagent in 1 ml serum-free medium;
c. Mix Solution A and Solution B together and incubate at room temperature for 20 minutes;
· Remove growth medium from the cells and add 4.5 ml serum-free medium to the cells;
· Add the transfection complex to the cells and incubate at 37oC for 4 hours.
· Add 0.65 ml FCS to each dish and incubate at 37oC overnight.
Note: Expression of the VSVG glycoprotein causes 293FT cells to fuse, resulting in the appearance of large, multinucleated
cells known as syncytia. This morphological change is normal and does not affect the production of the lentivirus.

DAY 3:

· Remove the medium from the cells and add 10 ml fresh medium to the cells.
·Incubate at 37oC for 24 hours.

Day 4:

· Collect viral supernatants* and add 10 ml fresh medium to the cells and incubate at 37oC for 24hours.

Day 5:

· Collect viral supernatants*.

*Clearing viral supernantant by centrifuging supernatants at 3000 rpm for 15 minutes at 4°C to pellet debris.
Optional: Filter the viral supernatants through a Millex-HV 0.45µm or an equivalent PVDF filter. Caution: Remember that you are working with infectious virus a□er viral supernantant collection. Follow the recommended guidelines for working with BL-2 organisms.

Determining the Lentiviral Titer with GFP Reporter Lentivirus

a. The day before transduction, plate cells at 30-50% confluence in 12-well plate and incubate cells overnight at 37°C.
b. On the day of transduction, prepare 10-fold serial dilutions of viral stocks in complete culture medium ranging from 1X to 106X.
c. Remove culture media from the cells and add 1ml of the dilutions directly to the cells.
d. If desired, add Polybrene® to each well to obtain a final concentration of 6µg/ml, and incubate at 37°C overnight.
e. The following day, replace the media-containing virus with fresh complete culture media.
f. After 2-4 days of transduction, observe the GFP expression under fluorescent microscope, and count the GFP positive cells to estimate the titer.

Lentivirus transduction andselection

a. One day before transduction, plate your target cells at 1 x 106 cells per well in a 6-well plate.
b. On the next day, mix 1.0 ml (or proper volume) of the lentiviral stock with 1.0 ml of complete medium and add to each well.
c. Add polybrene to each well to obtain a final concentration of 6µg/ml.
d. On the next day, replace the medium containing lentiviruses with complete culture medium.
e. A□er 3 days of transduction, replace the medium with fresh complete medium containing appropriate amount G418 for selection.
f. Replace medium containing G418 every 3-4 days for 3 weeks.
g. Pick up colonies for screening.

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