LIFESCT
Life Scientists' Service Center

Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
9610 Medical Center Drive, Suite 230, Rockville, MD 20850

Ready-To-Use Recombinant Lentivirus

Introduction

Lentivirus is a genus of viruses of the retroviridae family and has the unique ability among retroviruses of being able to infect both dividing non-dividing cells efficiently. Lentivirus is one of the most efficient vector to transduce mammalian cells for both *in vitro* and *in vivo*.

Important Guidelines for Transduction

- The optimal MOI (multiplicity of infection) is target cell specific. To determine the optimal MOI, it is advisable to perform several test transductions with reporter viral particles (e.g. LV-CMV-GFP or LV-CMV-mCherry) in different MOIs at 0.5, 2, 8, 20, 40 *etc*. The optimal MOI is then chosen to yield the highest percentage of successfully infected cells without invisible toxicity.

- Infection with the presence of hexadimethrine bromide (polybrene) at final 3~8 µg/ml in the cell culture medium enhances transduction of most cell types by 2~10 fold.

- The Infection Protocol for Suspension Cell is recommended for transduction of suspension cells and includes an additional spin step which concentrates the virus at the target cell surface to increase transduction efficiency.

- Transduction efficiency should be checked ~72 hrs following infection if no antibiotic selection is used.

- For cells that are more resistant to infection, it is desirable to select only the clones that stably express the transgene for downstream biological assays.

In-vitro Infection Protocol for Adherent Cell

1. Prepare Virus-containing Media

1.1 Thaw the lentiviral stock at RT.

1.2 Add desired amount of virus to growth media to achieve the desired optimal MOI. Lentiviral transduction unit (TU) to be used = MOI x number of cells to be infected. For example, if you intend to infect 50 thousands cells at MOI of 10, you need 10x50,000 = $5.0x10^5$ TU for the infection. If the original lentivirus stock is 10^9 TU/mL, then you will need 0.5 µl of the original stock for the dilution.

2. Infecting cells with adenovirus

2.1 Remove the original cell culture media.

2.2 Add the above lentivirus-containing media prepared from Step 1 to cell culture. Below is a general guideline for the amount of media used:

24-well plate: 0.2-0.3 mL
12-well plate: 0.5-0.8 mL
6-well plate: 1-1.5 mL/well
60 mm-plate: 3-4 mL/plate
10 cm-plate: 8-12 mL/plate

Optional: Add polybrene at final 3~8 µg/mL to cell culture medium enhances transduction of most cell types by 2~10 fold.

2.3 Incubate cells with the virus-containing media for overnight followed by removing virus-containing media and replacing with fresh, desired media next day after infection.

2.4 Check transduction efficiency 3~5 days after infection.

2.5 To create a stable cell line, add appreciate antibiotics 24~48 hrs post transduction.

In-vitro Infection Protocol for Suspension Cell

The following protocol has been given as a general starting point for 6-well plate. The transduction conditions shall be scaled up or down per the surface of tissue culture dishes.

- Resuspend the target cell to final 8 ml at density of ~10^6 cells/ml. Aliquot 2 ml into each of four 15 ml sterile conical tubes.

- Add appropriate volume of virus at optimal MOI to infect cells with the presence of polybrene at final 8 µg/mL. A reporter lentivirus like LV-CMV-GFP or LV-CMV-mCherry shall be included to monitor the transduction efficiency.

- Gently mix and incubate cells for 10 min at RT.

- Centrifuge cells for 30 min at 800× g at 32°C.

- Remove virus containing medium and resuspend cell pellet with 2 ml of fresh complete culture media followed by transfer of 2 ml cell suspension to a well of 6-well plate.

- Incubate cells for 24~72 hrs.

- Check transduction efficiency 3~5 days after infection.

- Split cells 1:3 or 1:5 and continue incubation for 48 hrs in complete media.

- The infected cells can then be selected for stable expression using appropriate antibiotic selection.

- Biological assays can be then carried out.

In-vivo Infection Protocol

The following protocol is given for stereotaxic gene delivery of lentivirus to the rodent brain.

Materials

- Super purified lentivirus in PBS, in vivo grade.
- Ethanol 70%.
- Mice or rats.
- Anesthetics and analgesics (e.g., ketamine *etc*).
- Sterile PBS
- Bone wax
- Triple antibiotic ointment

Procedures

1. Anesthetize and fix animal in stereotaxic apparatus.
2. Make incision and locate bregma.

FOR RESEARCH USE ONLY

LIFESCT
Life Scientists' Service Center
Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
9610 Medical Center Drive, Suite 230, Rockville, MD 20850

3. Surgery - Preparation of craniotomy by referring to the standard stereotaxic coordinates of mouse or rats. The craniotomy is drilled using a hand-held drill. The following table is an examples of stereotaxic coordinates.

Targeted region adult mouse brain00	Rostral (+) caudal (-)(mm)	Lateral (mm)	Ventral (mm)
Subthalamic nucleus	- 1.9	1.6	4.4
Dorsal hippocampus, CA1	- 2.1	2.0	1.4
Basolateral amygdala	- 1.5	2.75	4.75
Lateral ventricle	+ 0.5	0.75	2.5
Nucleus accumbens, core	+ 1.1	1.2	4.5

4. Injection of lentivirus for a single injection. Place the injection micropipette into the holder of the stereotaxic arm. Fill the micropipette with 3 µl lentivirus. Then slowly lower the micropipette to the desired z coordinate of the injection site. Apply pressure steadily via a pump or syringe to inject 100~500 nl lentivirus at >1E+9 TU/ml within 60~90 seconds.

5. After recovery of animal, feed the animal as usual until the transgene expression is checked one week after lentivirus injection.

Note: If lentivirus is administrated to mouse via different routes, please refer the lentivirus injection dose to the following table.

Lentivirus Administration Route	Dose (TU)
Intracerebral	$5x10^5$~$5x10^6$
Intraventricular	10^6~$5x10^6$
Intravenous	~$5x10^8$
Intranasal	10^7~$3x10^7$
Intravitreal	10^6~$4x10^6$