Protocol ● Lentifect™ Lentivirus

Note: This protocol is intended to be a general guideline only. Different cell lines will have different requirements for growth media composition and multiplicity of infection (MOI). Where applicable, use the conditions that are optimal for your particular cell line.

I. Getting Started

Storage of lentivirus

Upon receipt of a new lentivirus preparation, place the tubes in storage at -80 °C immediately. Avoid repeated freeze-thaw cycles, which can result in a loss of infectivity. If necessary, tubes of Lentifect™ lentivirus can be divided into smaller aliquots upon first thawing and placed at -80 °C.

Condition of cells

Always use healthy cells that are well maintained and passaged regularly. Make sure the culture is free from bacteria, fungi, or *Mycoplasma* contamination. If the cells are from a recent liquid nitrogen stock, passage the cells at least 2 times before transfection.

Multiplicity of infection

Before transducing with Lentifect™ lentivirus, it is essential to know the MOI for your cell line, which is the optimal number of virus particles to infect each cell with. The optimal MOI is known for many cell lines. Refer to the table below for a list of MOIS for many common cell lines. If you are unable to locate the known MOI for your cell line, GeneCopoeia recommends determining the MOI by first transducing your cells with lentivirus expressing a fluorescent reporter such as GFP or mCherry.

Cell line	Tissue	Cancer/cell type	Species	MOI
A431	Epithelial	Carcinoma	Human	5
A549	Lung	Carcinoma	Human	5
Astrocytes	Nervous system	Primary	Human	1
B16-F10	Epithelial	Melanoma, metastatic	Mouse	5
BMM	Bone Marrow	Primary	Human	8
BxPC-3	Pancreas, epithelial	Adenocarcinoma	Human	10
H3255	Lung	Carcinoma, NSCLC	Human	10
HCT116	Colon	Carcinoma	Human	5
HeLa	Cervix	Carcinoma, epitheloid	Human	3
HEK293T	Kidney	Tumor	Human	5
Hepa1-6	Liver	Carcinoma	Mouse	3
HMVEC	Endothelial	Endothelial, microvascular	Human	100
HT-29	Colon	Adenocarcinoma	Human	3

HUVEC	Umbilicus	Endothelial cells	Human	**100**
Jurkat	Blood	Leukemia, Acute T Cell	Human	**10**
LLC-1	Lung	Carcinoma	Mouse	**6**
LNCaP	Prostate	Carcinoma	Human	**5**
MM200	Skin	Melanoma	Human	**5**
MCF-7	Breast	Adenocarcinoma	Human	**2**
MDA-MB-231	Breast	Adenocarcinoma	Human	**1**
MM-AN	Skin	Melanoma, metastatic	Human	**16**
MMC	Breast	Carcinoma	Mouse	**4**
MRC-5	Lung, embryonic	Fibroblasts	Human	**1**
NB4	Blood	Leukemia, acute promyelocytic	Human	**10**
PC12	Adrenal gland	Pheochromocytoma	Rat	**20**
SKOV-3	Ovary	Adenocarcinoma	Human	**15**
U-2 OS	Bone	Osteosarcoma	Human	**5**

Table 1. Lentiviral particle MOIs for some commonly used cell lines.

II. Transduction of Target Cells with Lentivirus

The transduction efficiency depends upon the target cells and experimental procedure.

Day 1: Plate cells

1. Plate 2–10 x 10^4 of the target cells per well in a 24-well plate 24 hours prior to viral infection. Use 0.5 ml of cell specific medium supplemented with 5% heat-inactivated fetal bovine serum, and penicillin-streptomycin (optional) for each well. Incubate the cells at 37°C with 5% CO_2 overnight.

Note:
Make sure the cells reach 70-80% confluence at the time of transduction. The actual cell number to be plated depends on the cell type.

Day 2: Transduce target cells

2. For each well, prepare 0.5 ml of virus suspension diluted in complete medium with Polybrene (Millipore Sigma) at a final concentration of 5–8 µg/ml.

Note:

Use several dilutions of pseudoviral stock (0.1 µl to 100 µl). We recommend gradient dilutions of 0.1 µl, 0.3 µl, 0.5 µl, 0.7 µl, 0.9 µl for purified particles. In addition, we recommend including a transduction with the eGFP control and other appropriate positive and negative controls. Mix the virus with the medium gently by inverting the tubes several times. Do not vortex.

3. Infect the target cells by removing the old culture medium and replacing it with 0.5 ml of diluted viral supernatant. For one well (mock well control), add 0.5 ml of complete medium with Polybrene. Place the plates in a 37°C incubator with 5% CO_2 and incubate cells overnight. (Optional: Place the plates for 2 hours at 4-8°C; then transfer the plates to a 37°C incubator with 5% CO_2 and incubate cells overnight.)

Note:

Incubating cells with lentivirus for 2 hours at low temperatures can significantly increase the transduction efficiency. However, this step may be omitted if the cells cannot tolerate low temperatures.

Day 3: Replace medium/Split cell culture

4a. Replace the old medium with 0.5 ml of fresh complete medium (without Polybrene). Continue to 5a.

4b. Alternatively, split the cells 1:5 to 1:25 depending on the cell type by trypsinizing and re-seeding the cells onto 6-well plates or 10-cm culture dishes. Continue incubating for 48 hours in cell-specific medium. Continue to 5b.

Day 5: Analyze transduced cells or start drug selection of stably transduced cells

5a. The infected target cells can be analyzed for transient expression of transgenes using an appropriate biological assay. If you have used an internal eGFP control, determine the percentage of infected cells by counting fluorescing cells by flow cytometry or with a fluorescent microscope.

5b. To select stably transduced cells, replace old medium with fresh complete medium containing the appropriate selection drug every 3–4 days until drug-resistant colonies become visible (generally 7–14 days after selection).

GeneCopoeia, Inc.
9620 Medical Center Drive, #101
Rockville, Maryland 20850
Tel: 301-762-0888 Fax: 301-762-8333
Email: inquiry@genecopoeia.com
Web: www.genecopoeia.com