STANDARD OPERATING PROCEDURE

In Vivo Imaging Reagents

RediFect Lentiviral Particles Transfection Protocol

I. Infection of Target Cells

Materials

- 1 vial RediFect Lentiviral Particles (Part #: CLS960002, CLS960003, or CLS960004)
- Hexadimethrine Bromide
- Complete medium containing 10% fetal bovine serum
- 24 well cell culture treated plates
- 96 well cell culture plates
- 96 well black cell culture plates
- Mammalian cells to be transduced

During the infection procedures, wear mask and sleeve covers.
All decontamination procedures should be performed with fresh 10% bleach.

Day 1: Plate 50,000 wild-type cells per well in complete medium (please check your cell growth medium condition to ensure cell viability) into wells of a 24 well plate (for cell culture). Incubate cells for 24 hours.

Day 2: Thaw a vial of RediFect lentiviral particles and place on ice. Replace culture medium with 500ul of fresh complete medium containing hexadimethrine bromide (polybrene*) at a final concentration of 4 ug/ml.

* Polybrene enhances transduction of most cell lines. However, some cells such as mesenchymal stem cells or primary neurons are known to be sensitive to polybrene. If your cells are sensitive, do not add polybrene, and the cells should still be transduced.

Perform two MOI dose trials where viral particles are added directly to the cells at MOIs (Multiplicity Of Infection) according to the following:

- First Trial: 0 and 50
- Second Trial: 0 and 100

Incubate both MOI dose trials for 24 hours

0 = Control dose (no viral particles added)

To determine the total number of Particles to use, multiply the number of cells by the desired MOI.

The total media volume should be 500ul to 1000ul.

If the optimal MOI is unknown for the cell line of choice, it is recommended to use a range of MOIs (20-100).

Day 3: Discard the medium which contains virus (decontaminate medium during disposal) and gently wash the cells with fresh medium. Replace the medium with 1ml of fresh pre-warmed complete culture medium and incubate cells for 24 hours. (For puromycin selection, see Section III of this document. Puromycin concentration should be optimized for each cell line; typical concentrations range from 2-5 µg/mL. ***CSL960003 Red-FLuc-GFP does not have puromycin resistance***).

Note: Puromycin is stable for up to 3 months at room temperature and at least 1 year at 4°C.
For optimal stability and long term storage, aqueous solution aliquots can be stored at -20°C.

Day 4: Passage the cells to a new 24 well plate with 10,000 cells per well. Add fresh complete culture medium for a total volume of 1ml per well. Passage the control group (MOI of 0) according to the same procedure.

Day 6: Repeat passaging procedure from Day 4. (Save 1 or 2 small flasks or 12 well plate of infected cells as backup if needed).

Day 8: Seed 5,000 and 1,000 cells/well in media on black cell culture plate.

Day 10 and forward: Perform *in vitro* assay for bioluminescence signal from the expression of Red-Fluc luciferase (black cell culture plate) as follows:

Seed 5, 50, 500, 5000 cells/well. Incubate at 37°C for 5-10 minutes before performing bioluminescent imaging. For *in vitro* bioluminescence assay, final concentration of D-luciferin is 150µg/mL in the media. Image cells (10 to 30 minutes in an IVIS) noting the peak imaging time.

Lentiviral driven GFP has relatively low expression.

Once a good signal is confirmed (>100 photons/cell/sec), seed 50K and 100K cells per well in triplicates, and check the bioluminescence signal at 0, 1, 2, 3, and 4 weeks.

Freeze backup cells and original cells as 1st generation infected cells.

Check cell viability: After 2-4 weeks, thaw a frozen vial of 1st generation infected cells and perform *in vitro* assay for expression of luciferase to ensure stable transfection of healthy stock.

II. Generation of New Red-Fluc Cell Lines

To generate heterogeneous population of infected cells

1) Plate cells in two cell culture plates at 130 cells per plate in 150 ul of media per well (about 1-2 cells per well). Change media 2 times per week. Mark wells that display good cell growth. Screen wells for duplicates and mark duplicate wells. Incubate cells for 2-8 weeks until they become confluent.

2) Split cells 1:1. Image one plate to determine BLI positive clones, and expand the positive clones from the second plate. Passage cells with flasks, starting from small size flasks. Expand the cells and collect in vials with 2 million cells per vial for freezing/storage as backup.

3) Check the cell viability and *in vitro* assay for expression of luciferase. Choose clones that display the same morphology as the parental culture.

4) Compare the cell growth pattern of parental cells with new red-Fluc cells. After confirmation of the morphology and bioluminescence signal *in vitro*, continue to test *in vivo* growth and signal stability using *in vivo* models.

III. Puromycin Titration Protocol (kill curve)

****For puromycin resistant cells only*. DO NOT perform with Red-Fluc-GFP cell lines (CLS960003), as these do not have puromycin resistance.****

1) Plate 10000 cells into wells of a 96-well plate with 100 ul fresh media.

2) The next day add 500–5,000 mg/ml of puromycin* to selected wells.

3) Examine cell viability every 2 days. Keep a record of survival percentage of each puromycin dose.

4) Culture for 10–14 days. Replace the media containing puromycin every 3 days.

* Use the minimum concentration of puromycin capable of causing cell death within 3–5 days for your selected cell type.

For research use only. Not for use in diagnostic procedures

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com