shRNA Lentiviral Particles Transduction

DAY 1

- Plate target cells in a 12-well plate 24 hours prior to viral infection.
- Add 1 ml of complete optimal medium (with serum and antibiotics) and incubate cells overnight. The cells should be approximately 50% confluent on the day of infection (Day 2).

NOTE: It is possible to use other plate formats for transduction as well. In this case, the amount of cells should be adjusted depending on the growth area of the well or plate.

DAY 2

- Prepare a mixture of complete medium with Polybrene® (sc-134220) at a final concentration of 5 µg/ml.
- Remove media from plate wells and replace with 1 ml of this Polybrene/media mixture per well (for 12-well plate).

NOTE: Polybrene is a polycation that neutralizes charge interactions to increase binding between the pseudoviral capsid and the cellular membrane. The optimal concentration of Polybrene depends on cell type and may need to be empirically determined (usually in the range of 2-10 µg/ml). Excessive exposure to Polybrene (>12 hr) can be toxic to some cells.

- Thaw lentiviral particles at room temperature and mix gently before use.
- Infect cells by adding the shRNA Lentiviral Particles to the culture.
- Swirl the plate gently to mix and incubate overnight. The amount of viral particles to use varies greatly depending on the characteristics of the cell line used.

NOTE: Keep thawed shRNA Lentiviral Particles on ice. Repeated freeze-thaw cycles and prolonged exposure of the particles to ambient temperatures may result in decreased viral titers.

NOTE: When transducing a shRNA lentiviral construct into a cell for the first time we suggest using several amounts of shRNA lentiviral particle stock. In addition, we recommend to include one well with cells transduced with Control shRNA Lentiviral Particles (sc-108080).

DAY 3

- Remove the culture medium and replace with 1 ml of complete medium (without Polybrene).
- Incubate the cells overnight.

DAY 4

- To select stable clones expressing the shRNA, split cells 1:3 to 1:5, depending on the cell type, and continue incubating for 24-48 hours in complete medium.

DAY 5-6 and forward

- Select stable clones expressing the shRNA via Puromycin dihydrochloride (sc-108071) selection.
- For puromycin selection, use an amount sufficient to kill the non-transduced cells. Puromycin concentrations ranging from 2 to 10 µg/ml are usually sufficient, but a puromycin tritation is recommended when using a new cell line.
- Replace medium with fresh puromycin-containing medium every 3-4 days, until resistant colonies can be identified. Pick several colonies, expand them and assay them for stable shRNA expression.

NOTE: Resulting puromycin-resistant clones may have varying levels of shRNA expression due to the random integration of the lentiviral construct into the genome of the cell.

NOTE: For shRNA expression analysis by Western Blot, prepare cell lysate as follows:

- Wash cells once with PBS.
- Lyse cells in 100 µl of a 1:1 mixture of 2x Electrophoresis Sample Buffer (sc-24945) and RIPA Lysis Buffer (sc-24948) by gently rocking the 12-well plate or by pipetting up and down.
- Sonicate the lysate on ice if necessary.

NOTE: For shRNA expression analysis by RT-PCR, isolate RNA using the method described by P. Chomczynski and N. Sacchi (1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162: 156-159) or a commercially available RNA isolation kit.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

shRNA LENTIVIRAL PARTICLES SUPPORT REAGENTS

PRODUCT	CAT. #	DESCRIPTION	AMOUNT
Control shRNA Lentiviral Particles	sc-108080	Control shRNA Lentiviral Particles is available as an alternate negative scrambled shRNA sequence control.	200 µl
copGFP Control Lentiviral Particles	sc-108084	copGFP Control Lentiviral Particles are provided as transduction-ready viral particles.	10-20 transductions
Electrophoresis Sample Buffer	sc-24945	Ready-to-use reducing electrophoresis sample buffer solution for the preparation of protein samples to be separated in SDS-PAGE.	25 ml; 2X concentrate
RIPA Lysis Buffer	sc-24948	For use in mammalian cell lysis; with protease inhibitors. Available in four vials: 1X lysis buffer, PMSF, protease inhibitor cocktail and sodium orthovanadate.	50 ml
Puromycin dihydrochloride	sc-108071	Available for selection and maintenance of cells transfected with the puromycin-N-acetyl-transferase (pac) gene.	25 mg
Polybrene®	sc-134220	Highly efficient infection reagent used to introduce retroviral vectors into mammalian cells.	1 ml