Lentiviral Activation Particles Transduction

PROTOCOL

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NOTE: This protocol is recommended for a single well from a 6-well tissue culture plate. Adjust cell and reagent amounts proportionately for wells or dishes of different sizes.

Day 1

- In a 6-well tissue culture plate seed 1.5 x 10^5 - 2.5 x 10^5 cells in 3 ml of standard growth medium per well, 24 hours prior to transfection (medium may contain serum and antibiotics). Grow cells to a 40–80% confluency. Initial cell seeding and cell confluency after 24 hours are determined based on the rate of cell growth of the cells used for transduction. Healthy and subconfluent cells are required for successful transduction with Lentiviral Activation Particles.

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 3 ml of this Polybrene® media mixture per well (for 6-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 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 Lentiviral Activation 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 cell for the first time we suggest using several amounts of Lentiviral Activation Particle stock. In addition, we recommend to include one well with cells transduced with **Control Lentiviral Activation Particles** (sc-437282).

NOTE: Use **copGFP Control Lentiviral Particles** (sc-108084) for measuring transduction efficiency.

Day 3

- Remove the culture medium and replace with 3 ml of complete medium (without Polybrene®).
- Incubate the cells overnight.

Day 4

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

NOTE: Cells must be maintained sub-confluent throughout the antibiotic selection described below.

Day 5 and Forward

- Select activated clones via **Puromycin dihydrochloride** (sc-108071), **Hygromycin B** (sc-29067) and **Blasticidin S HCl** (sc-495389) selection.
- For antibiotic selection, use an amount sufficient to kill the non-transduced cells.
- Puromycin concentrations ranging from 2–10 µg/ml, Hygromycin B concentrations ranging from 200–500 µg/ml and Blasticidin S HCl concentrations ranging from 1–20 µg/ml are usually sufficient, but a selective antibiotic titration is recommended for every cell line or cell type used.
- Replace medium with fresh selective antibiotic-containing medium every 3–4 days, until resistant colonies can be identified. Pick several colonies, expand them and assay them for stable target gene activation.

NOTE: Resulting selective antibiotic-resistant clones may have varying levels of target gene activation due to the random integration of the Activation constructs into the genome of the cell.

NOTE: For gene activation analysis by Western Blot, prepare cell lysate as follows:

- Change media to standard growth medium (without selective antibiotics) 3 days prior to cell lysis.
- To lyse adherent cells, aspirate media, rinse cells with PBS, scrape and centrifuge cells at low speed to obtain a cell pellet.
- To lyse suspension cells, transfer the culture to a centrifuge tube and centrifuge cells at low speed to obtain a cell pellet.
- Wash once with PBS and centrifuge again.
- For 100% confluent HEK 293 or HeLa cells, add 100 µl of **RIPA Lysis Buffer System** (sc-24948) to the pellet. For other cell lines or confluencies, the amount of RIPA Lysis Buffer System to use should be determined experimentally.
- Sonicate or shear cells.
- Incubate the lysate on ice for 10 minutes, vortex, and incubate again for 10 minutes on ice.
- Clear the lysate by centrifuging at high speed for 20 minutes at 4° C.
- Use the **BCA Protein Assay Kit** (sc-202389) to determine protein concentration.

NOTE: For gene activation 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 into genomic DNA of target cells.

References:
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