Lentiviral Labeling & Imaging

Size: 1 ml / vial

Storage Temperature: -70°C

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Protocol

Lentiviral Transduction.

This protocol may be used with 96-well, 48-well, 24-well, 12-well and 6-well plates.

Day 0: Seed cells at appropriate density.

Suggestion: Plate cells so that cell density will be ~25-50% confluent at the time of transduction.

Day 1: Transduction. Remove the medium from the tissue culture plate by aspiration and replace it with fresh complete medium containing 5-8 μg/ml polybrene. Gently mix lentivirus with 1ml pipette tip, and add appropriate amount of virus to each well.

Note: Transduction efficiency varies in different cell lines, and polybrene may be toxic to some cell lines.

Suggestion: Add 1-10 μl lentivirus per 1,000 cells. Spin transduction in a desktop centrifuge (e.g. Sorvall RT6000) at 1,000 × g for 60 min at room temperature helps increase of transduction efficiency.

Day 2: Replace the transduction medium with fresh complete medium to remove lentivirus and polybrene.

Note: Replace medium immediately after spin transduction if polybrene is toxic to the cells.

Day 3+: Observe cells under fluorescence microscope.

Safety Guidelines for Working with Lenti-miRNA.

The recombinant lentiviruses have been designated as Level 2 organisms by NIH and CDC. A Biosafety Level 2 (BSL-2) facility is required in order to work with lentiviruses. The information of Biosafety in Microbiological and Biomedical Laboratories (BMBL) can be downloaded from the following link:
http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf

Please be aware that you are working with media containing lentiviral particles which could transduce human cells.

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