

General Protocol for Lentiviral Transduction

Storage Temperature: -70°C

August 2013

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 1,000-5,000 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.

Suggestion: Lentiviral stock may be stored at 4 °C for up to 5 days. To reduce the number of freeze and thaw cycles, aliquot lentiviral stock to smaller tubes before storage at -80 °C.

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 3-4: Select transduced cells (>50% confluence is recommended) with medium containing appropriate antibiotics or by flow cytometry to sort out fluorescence-positive cells if necessary.

Note: The optimal antibiotic concentration varies from cell line to cell line.

Suggestion: A pilot experiment should be performed to determine the minimum concentration of antibiotic required to kill the untransduced cells before this experiment. For example, seed your model cells around 25-50% confluence in two 6-well plates and add 2 ml of complete medium containing various antibiotic concentrations to perform the antibiotic kill curve, change medium every 3 days. You should be able to determine the minimal antibiotic concentration within 7-14 days. Below are the suggesting concentrations for different antibiotic selection.

Puromycin (ug/ml): 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10.

Hygromycin (ug/ml): 0, 20, 40, 60, 80, 100, 150, 200, 300, 400, 500.

Blasticidin (ug/ml): 0, 1, 2, 4, 6, 8, 10, 12, 15, 20.

G418 (mg/ml): 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5.

Day 6+: Analysis of transduced cells.

Suggestion: Expand the stable cell lines and store the cell line stocks in liquid nitrogen.

Safety Guidelines for Working with Lentiviruses.

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.

User Notification

Biosettia products are sold to be used for research purposes only and may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Biosettia, Inc. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnosis, or therapeutics, or in humans. Products may be covered by pending or issued patents or may have certain limitations. Please contact Biosettia for more information. All prices and specifications are subject to change without prior notice. Product claims are subject to change. Please access the Biosettia online catalog for the most up-to-date information.

Biosettia warrants that all of its products will perform according to commercially reasonable standards. The company will replace, free of charge, any product that does not meet those specifications. Customer must notify Biosettia within thirty days of delivery. This warranty limits Biosettia's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Biosettia reserves the right to select the methods used to analyze a product unless Biosettia agrees to a specified method in writing prior to acceptance of the order.