



Lentivirus transduction protocols

1) Transduction Protocol for Adhesive cells :

Note: Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50 μ l of virus into each well of 24-well-plate where cell density is 50% to 90%. After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight.

Note: at the time of transduction, cells should be 50%-90% confluent. For example, seed HeLa cells at 0.5×10^5 /ml x 0.5ml in a well of a 24-well plate.

Day 1:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI (or to simplify it, add 50 μ l virus).
- Return cells to 37°C, CO₂ incubator.

Note: Try to avoid freezing and thawing. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

Day 3:

At ~72hr after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

Day 3 + (optional):

Sort transduced cells by FACS, and select for antibiotic resistance. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).



2) Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium in plate or in flash, in a CO² incubator if necessary.

Measure cell density. When density has reached ~3 x 10⁶ cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10⁶ cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more lentivirus or use the concentrated lentivirus in PBS solution).
- Grow cells in in a CO2 incubator.

Day 2-3:

At 48 to 72 hours after transduction, pass the cell into fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type (you need to test the killing curve for the antibiotic brand in your cell types). Continue growing cells in CO2 incubator.

Day 2-3:

At 48 to 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Note: Filter wavelength settings:

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|-------------------------------|---------|
| BFP filter: ~Ex380 | ~Em460; |
| CFP filter: ~Ex436 | ~Em480; |
| GFP filter: ~Ex450-490 | ~Em525; |
| YFP filter: ~Ex500 | ~Em535; |
| RFP filter: ~Ex545 | ~Em620; |
| iRFP filter: ~Ex690 | ~Em715 |

Attachment: GenTarget's Pre-made lentivirus Products:

| Product Category | Product Description (please click category name to see product's pages) |
|--------------------------------------|--|
| Human and mouse ORFs | Premade lentivirus expressin a human, mouse or rat gene with RFP-Blastididin fusion dual markers. |



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| Fluorescent markers | Preamde lentivirus express human codon optimized fluorescent protein, GFP / RFP/ CFP/ BFP / YFP . |
| Pathway Report lentivirus | Premade lentivirus express a fluorescent protein or a luciferase report under a pathway specific promoter. |
| Cell-specific Report lentivirus | Premade lentivirus express a fluorescent protein or a luciferase report under a cell specific promoter. |
| Luciferase expression | Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla with different antibiotic selection markers. |
| CRE recombinase | Premade lentivirus for expressing nuclear permeant CRE recombinase with different flurescent and antibiotic markers. |
| LoxP ColorSwitch | Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo. |
| CRISPR /hu CAS9 | Preamde lentivirus express humanized wild-type Cas9 endonuclease for genomic editing with CRISPR |
| TetR inducible expression repressor | Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system. |
| iPS factors | Premde lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLF4) factors with different fluorescent and antibitoic markers |
| T-antigen Expression | Express different large and small T antigen with different selection markers |
| Cell Organelle imaging | Premade lentivirus for cell organelle imaging. The fluorescent marker GFP/RFP/CFP was sub-cellular localized in different cell organelle for living cell imaging. |
| LacZ expression | Express different full length β- galactosidase (lacZ) with different selection markers |
| Anti-miNA lentivirus | Pre-made lentivirus expression a specific anti-miRNA cassette. |
| Fluorescent-ORF fusion | Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target. |
| Pre-made shRNA lentivirus | Premade shRNA lentivirus for knockdown a specific genes (P53, LacZ, Luciferase and more). |
| microRNA and anti-microRNA lentivirus | Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and mouse miRNA. |
| Negative control lentiviruses | Premade negative control lentivirus with different markers : serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression |



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| | effects. |
| Other Enzyme expression | Ready-to-use lentivirus, expressing a specific enzymes with different selection markers. |