



Lentivirus transduction protocols (for reference only)

1. Adhesive cells Transduction Protocols:

Day 1: Remove the culture medium. Add fresh, warmed, complete medium (0.5ml). Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Return cells to 37°C/CO² incubator. (Try to avoid thaw and freeze cycles for pre-made lentivirus. But if you cannot use all virus in one time, you still can re-freeze the virus at -80°C for future use. But virus titer will decrease by ~10% for each re-thaw.)

Day 3: At ~72hr after transduction, check the transduction rate *via* fluorescence image with a suitable filter under fluorescent microscope, or calculate the exact transduction rate via Flow Cytometry System (FACS) or any flow cytometry (such as Guava machine).

Day 3 + (optional): Transduced cells can be sorted out via FACS, selected by its specific antibiotics. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line. (Refer to any literatures about How to generate stable cell lines.)

Note 1: **A quick application protocol is:** add 50ul virus into one well in 24-well-plate where cell density is at 50% ~ 75%. At 72 hours after virus added (no need to change medium), visualize the positive rate under fluorescent microscope. For stable cell line generation, pass cell into antibiotic containing medium, or sort the cells via fluorescent signal. Then , select the cells by antibiotics.

Note 2: For some cell types such as primary cells – It may take up to longer time for maximal expression; in some cases, maximal expression may not be detected until 1 week post-transduction.

2. Suspension cells transduction Protocols:

- Grow your cell in your completed suspension culture medium, shaking in flask in CO² incubator if necessary;
- Measure cell density. When cell grow to ~3 x 10⁶ cell/ml, measure cell viability (should be > 90%), then diluted cells into 1 x 10⁶ cell/ml in completed medium;
- Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **50ul or 100ul virus per 0.5 ml**



cell (Note: depending on the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO² incubator.

- At 24 hours after transduction, add equal amount of fresh medium containing related antibiotics (Note: each particles contain an antibiotic marker and the antibiotic amounts to use depends upon cell types). Grow in CO² incubator.
- At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).
- You can sort the fluorescent positive cells, and maintain the antibiotic selection to generate stable cell lines.

Note: Filter wavelength settings:

GFP filter:	~Ex450-490	~Em525;
RFP filter:	~Ex545	~Em620;
CFP filter:	~Ex436	~Em480;
YFP filter:	~Ex500	~Em535; (has overlapped spectrum with GFP)