



1. Adhesive cells Transduction Protocols:

Day 0: Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 10% ~50% confluent.)

For example, seed Hela cells at $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ in a well of a 24-well plate;

Day 1: Remove the culture medium from the cells. Add fresh complete medium (Note: use as little media as possible at transduction). 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.

For example, add 5ul ~ 50ul of lentiviral stock to the cells in 24-well plate above (getting MOI from 0.5 to 5).

Day 3: At the time of ~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 Quava machine).

Note: 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.

Day 3 + (optional): Transduced cell can be sorted out via FACS. Or you can select transduced stable cell line by Blastcidin. A pilot experiment should be done to determine the kill curve for your specific cell line, Bsd ranged from 0.5ug ~10ug/ml.

2. Suspension cells transduction Protocols:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO₂ incubator;
2. Measure cell density. When cell grow to $\sim 3 \times 10^6$ cell/ml, measure cell viability (should > 90%), then diluted cells into 1×10^6 cell/ml in completed medium;
3. Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **200ul virus per 2ml cells** (Note: depend upon the cell types; you may need to use more viruses). Grow cells in flask, shaking in CO₂ incubator.
4. At 24 hour after transduction, add equal amount of fresh medium containing final concentration of Blastcidin at 5 ~ 10ug/ml depend upon cell types. Grow cell shaking in CO₂ incubator. (Note: Gentarget's premade lentivirus contain Blastcidin resistance. So add Blastcidin antibiotic will enrich only the transduced cells for maximum protein production.)
5. At at 72 hours, Check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).
(Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620).