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Spinoculation of Suspension Cells

This protocol is for transduction of suspension cells (Jurkat T cells, PBMC, PBL, B cells etc.) with lentivirus. To obtain the desired infection rate (low and high MOI; multiplicity of infection) in your target cells, serial titration of virus and cell numbers are required. Concentration of virus may be required for difficult to transduced cells such as primary cells. Infection volume can be scaled down to 12, 24, 48, and 96 well plate depends on your cell number and viral supernatant volume. The following protocol is for a 6 well plate.

1. Quick thaw virus in a 37°C water bath.
2. Wash cells to be transduced (typically 5-20 million cells per well) in PBS.
3. Resuspend cells at 5-20 million per 0.5 ml in complete culture media.
4. Transfer 0.5ml of resuspended cells per well and add 4.5 ml virus per well. Tilt plate to mix.
5. Add 50 µl of 1M HEPES, pH 7.4 (10µl/ml) and 1X TransDux (SBI Cat #LV850A-1) per well. Tilt plate to mix.
6. Seal plate with plate sealer and press down evenly. Cover plate.
7. Spin in balanced clinical tabletop centrifuge for 1.5-2 hours at 2,400 rpm at 32°C.
8. Carefully remove plate sealer. Avoid any splashing.
9. Resuspend cells in the viral supernatant and recover (scrape) cells off from the bottom of the plate. Transfer cells to conical tube.
10. Spin cells at 1,500 rpm 5 min. and aspirate media.
11. Resuspend cells in culture media at a density of 50,000 cells/ml and continue to culture for desired incubation time.
12. Check the infection rate by FACS analysis.

For long term storage of Jurkat stable cell line, cells can be stored in liquid nitrogen in freezing media (90% FBS and 10% DMSO) followed by standard cell freezing method.