**SOP for the Infection of Suspension Cells with Lentiviral or Retroviral Supernatant.**

1. On the day of infection, pellet 1x10^6 suspension cells.
2. Resuspend cell pellet in 1 mL virus + 1 uL 4mg/ml polybrene.

*(Note regarding T-cell infection: T cells can be very sensitive to polybrene and a lesser concentration of polybrene may be required. We recommend 1ug/ml.)*

1. Add cells to a 12 well plate. Seal plate and place in a microtiter rotor and spin at 1800 rpm for 45 minutes at room temperature.

*(Note: Spinning cells at 1800 rpm for 45 minutes is not enough to sediment free virus. It is thought that virus on membrane fragments is spun onto cells in a manner which effects greater infection.)*

4.) Return plate to incubator and incubate for 3-6 hours at 37°c / 5% CO2.

1. Add 1 ml normal target cell media for a total volume of 2 ml. Incubate overnight at 37°c.

6.) After infection, centrifuge the cells (500 x g for 5 minutes) and resuspend in the appropriate media for normal growth of the target cells. Allow the cells to grow in a T75 flask at 37°c for an additional 24-48 hours before puromycin selection. (50 -4000ng/ml\*\* puromycin in normal target cell complete medium.)

Replace media with puromycin selection media every 3-4 days.

\*\* cell type, infection with virus, transfection with plasmid, cell health, culture medium, and growth conditions can all effect the optimal puromycin concentration for selection. It is highly recommended that you first perform a puromycin titration (kill curve) to determine the lowest puromycin concentration that begins to cause massive cell death within 3-4 days.