Cell Based Lentiviral Titer
Last Updated: 12/19/12

**IMPORTANT**
Follow approved safety protocol for working with lentiviruses

NOTES: This protocol is for a cell-based lentiviral titer. Always titer frozen virus (not fresh) because you lose ~half of your titer after every freeze/thaw (i.e. you will be thawing a previously made virus for your actual experiments).

REAGENTS: 293T Growth Media
DMEM (Gibco) + 10% FBS (Heat Inactivated)
NOTE: do NOT use antibiotics in the growth media

PROTOCOL:

Day 1  Seed 293T Cells
1 Seed 200K 293T cells per well of a 6-well plate
   Note: 3 wells will be used for each virus being titered plus 2 extra wells for the cell count, and 1 uninfected well to setup FACS.
   i.e.  count  count  mock
         1:10000  1:1000  1:200

Day 2  Count and Transduce 293T Cells
1 Change media to 1mL fresh 293T growth media per well
2 Trypsinize, count, and average the total # of 293T cells in 3 wells.
3 Thaw frozen virus on ice (usually take 30+ minutes)
4 Dilute frozen virus 1:10 in 293T media
5 Add 1uL, 10 uL, and 50uL of diluted virus to 3 separate wells
6 Mix and incubate at 37C + 5% CO2

Day 3  Media Change
1 Aspirate (by hand) virus containing media and place in bleach
2 Add 2mL fresh 293T growth media
3 Incubate at 37C+5% CO2

Day 5  FACS Analysis and Calculation of Viral Titer
1 Aspirate media from each well and wash 1X with PBS
2 Add 1mL trypsin to each well, incubate until cells release from plastic, and pipette to make single cell suspension
3 Add 2mL HANKS Buffer + 2% FBS to inactivate trypsin and transfer to 15 mL conical tube
4 Pellet cells for 3 minutes at 1100 RCF
5 Aspirate supernatant and resuspend in 500 uL HANKS + 2% FBS
6 Follow standard protocol to prepare samples for FACS
7 Analyze % Transduced (% GFP positive) cells with a FACS scanner
8 Calculate viral titer:
   IU/mL= [cell number x (% fluorescent cells / 100)] x Dilution factor

Notes: cell number = cells per well before transduction (from Day 2)
Dilution factor = 10,000 (1uL virus), 1,000 (10uL virus), or 200 (50uL virus)
For the most accurate viral titer calculations, only use conditions that result in fewer than 15% Transduced cells.