1. Purpose
   1.1. The purpose of this protocol is to determine the titer of a retroviral vector.
   1.2. This procedure is routinely performed in the Vector Development Laboratory (VDL) following Good Laboratory Practices (GLP).

2. Abbreviations and Definitions
   2.1. SOP    Standard Operating Procedure
   2.2. VDL    Vector Development Laboratory
   2.3. BSC    Biological Safety Cabinet
   2.4. BSL-2  Bio-safety Level 2
   2.5. GLP    Good Laboratory Practice
   2.6. DMEM   Dulbecco’s Modified Eagle’s Medium
   2.7. Pen/Strep/anti-mycotic Penicillin/Streptomycin/anti-mycotic Solution
   2.8. FBS    Fetal Bovine Serum
   2.9. Trypsin 0.25% Trypsin-EDTA
   2.10. PBS   Phosphate Buffer Saline
   2.11. Complete Medium DMEM with 4 mM L-glutamine, 4.5 g/L glucose, adjusted to contain 1.5 g/L sodium bicarbonate, 10% FBS, and 1% Pen/Strep/anti-mycotic
   2.12. TU    Transducing Units

3. Equipment, Materials, and Reagents
   NOTE: All materials in contact with cells must be sterile, pyrogen-free and used according to the manufacturer’s directions unless stated otherwise. Equivalent materials and equipment may be used but all changes must be recorded.

3.1. Equipment
   3.1.1. BSC
   3.1.2. 37°C/5% CO₂ incubator
   3.1.3. Pipette aid
   3.1.4. Inverted microscope with 20X objective
   3.1.5. Table top centrifuge

3.2. Materials
   3.2.1. 5 mL snap cap tubes  Falcon
   3.2.2. 100 mm cell culture dishes  Corning
3.2.3. 6-well plates         Corning
3.2.4. Serological pipets    Corning
3.2.5. Sterile pipet tips    VWR
3.2.6. 1.7 ml micro-centrifuge tubes Axygen

3.3. Reagents
3.3.1. Complete medium       VDL
3.3.2. DMEM                  Hyclone
3.3.3. FBS                   Atlas
3.3.4. Pen/Strep/anti-mycotic Cellgro
3.3.5. Trypsin               Cellgro
3.3.6. PBS                   Invitrogen
3.3.7. Trypan blue           Sigma
3.3.8. G418                  Invitrogen
3.3.9. Polybrene®            Invitrogen
3.3.10. Crystal Violet       Sigma

3.4. Starting Materials
3.4.1. Retrovirus containing supernatant from transfection of packing cells
3.4.2. One 150mm dish of confluent NIH3T3 cells (murine fibroblast)

3.5. Test Sample Identification
3.5.1. The bar code on the plasmid will be scanned and compared to the computer database to ensure the correct sample is being processed.
3.5.2. One copy of the barcode will be printed and applied to a copy of this SOP for the final record.

4. Procedure
Note: All procedures are performed in a BSC.

Preparation of NIH3T3
4.1. Remove NIH3T3 cells from incubator and transfer to BSC.
4.2. Remove media and wash cells once with PBS.
4.3. Remove PBS and trypsinize cells.
4.4. Count cells and plate 0.5-1x10^5 cells per well in a 6-well plate.
4.5. Incubate overnight in a 37°C/5% CO₂ incubator.

Transduction
4.6. Prepare 40 mL of culture medium and add 120 µL of 4 mg/mL polybrene.
4.7. **Note:** Polybrene is a polycation that reduces charge repulsion between the virus and the cellular membrane.

4.8. Collect virus-containing medium from packaging cells.

4.9. Filter the medium through a 0.45-µm cellulose acetate or polysulfonic filter.

4.10. **Note:** Do not use a nitrocellulose filter because nitrocellulose binds proteins in the retroviral membrane and destroys the virus.

4.11. Prepare six 10-fold serial dilutions as follows:

4.11.1. Add 2.70 mL of medium containing polybrene to each of six micro-centrifuge tubes.

4.11.2. Add 300 µL of virus-containing medium to the first tube.

4.11.3. Transfer 300 µL of virus-containing medium from tube 1 to tube 2. Continue serial dilutions by transferring 300 µL of each successive dilution to the next prepared tube.

4.12. Infect NIH3T3 cells by adding 1 mL of the diluted virus medium to the wells.

**Note:** Each dilution is assayed in duplicate to ensure accuracy.

**Note:** The final polybrene concentration will be 4 µg/mL in 3 mL.

4.13. Incubate overnight in a 37ºC/5% CO2 incubator.

4.14. At 24 hours post-infection, remove the medium and replace with complete medium containing 1 mg/mL G418.

4.15. Replace the medium with fresh medium containing G418 every 3-4 days.

4.16. After 7-10 days of selection, discrete antibiotic resistant colonies in one or more of the dilution wells should be visible. Remove the medium and wash the cells twice with PBS.

4.17. Add crystal violet solution and incubate for 10 minutes at room temperature.

4.18. Remove the crystal violet solution and wash the cells twice with PBS.

**Quantification and Calculation of Titer**

4.19. To calculate the titer, count the number of stained colonies. Then use the following formula to determine the titer (TU/ml) of the viral stock.

\[
\text{Number of discrete colonies/ dilution factor} = \text{TU/mL}
\]

**Sample calculation:**

An average of 50 plaques formed in the 1:10,000 dilution wells

\[
50/0.0001 = 2.5 \times 10^8 \text{ TU/mL}
\]

5. **Data Collection and Management**
5.1. All data will be collected on the Retrovirus Titer Worksheet and the results will be transferred to a laboratory notebook.

5.2. The final titer and date the titering protocol was complete will be recorded on the Lab Meeting Sheet.

5.3. Deviations of the protocol will be recorded in the laboratory notebook and on the Lab Meeting Sheet.

6. **Review and Revisions**

Written by: ________________________
Director, VDL

Reviewed by: ________________________
Director, QA/QC

Reviewed by: ________________________
Director, Vector Production

Date Issued: 1/30/2006 Replaces VDL 607.0

**Annual Review:**

2011

Reviewed without changes Changed and this version archived

Reviewed by: ________________________
QA/QC by: ________________________

Date Issued: 6/8/2011 Replaces VDL 607.1

2012

Reviewed without changes Changed and this version archived

Reviewed by: ________________________
QA/QC by: ________________________

Date: ________________________

2013

Reviewed without changes Changed and this version archived