VDL602.2 RAPID ASSAY FOR DETERMINING ADENOVIRAL VECTOR TITER

1. Purpose

1.1. The purpose of this protocol is to determine the number of infectious adenoviral particles.
1.2. The starting material is purified adenoviral vectors.
1.3. This protocol is based upon the Adeno-X™ Rapid Titer Kit (BD-Bioscience-Clontech).
1.4. 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. GLP Good Laboratory Practice
2.5. Antibiotics Pencillin/Streptomycin/Anti-Mycotic
2.6. DMEM Dulbecco’s Modified Eagle’s Medium (4500mg/mL Glucose, 4.0mM L-Glutamine) with 1% antibiotics
2.7. FBS Fetal Bovine Serum
2.8. DMEM10 DMEM with 10% FBS
2.9. Trypsin 0.25% Trypsin-EDTA
2.10. HRP Horseradish Peroxidase
2.11. DAB Diaminobenzidine
2.12. PBS Phosphate Buffer Saline
2.13. BSA Bovine Serum Albumin
2.14. PBS/BSA PBS with 1% BSA

3. Equipment, Materials, and Reagents

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

3.2. Materials

3.2.1. 12-well dish Corning
3.2.2. Serological pipets Corning
3.2.3. Sterile pipet tips VWR
3.2.4. 1.7 ml tubes Axygen
3.2.5. Pasteur pipets  

3.3. **Reagents**

3.3.1. DMEM  
Hyclone

3.3.2. DMEM10  
VDL

3.3.3. PBS  
Invitrogen

3.3.4. PBS/BSA  
VDL

3.3.5. BSA  
Sigma

3.3.6. FBS  
Atlas

3.3.7. Antibiotic  
Cellgro

3.3.8. Adeno-X™ Rapid Titer Kit  
BD Bioscience – Clontech

3.3.9. Methanol

3.4. **Starting Materials**

3.4.1. One 12 well plate of 80% confluent 293 cells

3.4.2. Purified adenoviral vector, 10 μL

3.4.3. Adeno-X™ Rapid Titer Kit

4. **Procedure**

**Infection of cells**

4.1. Aspirate media from cells and replace with 1 mL DMEM10.

4.2. Using PBS or DMEM10 as diluent, prepare 10-fold serial dilutions (10⁻² – 10⁻⁸/mL) of adenoviral vector.

4.3. Add 100 μL of adenoviral dilutions 10⁻⁶, 10⁻⁷ 10⁻⁸ dropwise to each well.  
**Note:** Each dilution of virus should be assayed in duplicate to ensure accuracy.

4.4. Incubate cells in the 37°C/5% CO₂ incubator for 48 hours.

**Fixation of cells and Immunostaining**

4.5. Aspirate medium and allow cells to dry in BSC for 5 minutes.

4.6. Fix cells by very gently adding 1 mL ice-cold methanol to each well.

4.7. Incubate the plate at -20°C for 10 minutes.

4.8. Aspirate methanol and gently rinse wells three times with 1 mL PBS/BSA.

4.9. Dilute mouse anti-hexon antibody 1:1000 in PBS/BSA.

4.10. Aspirate final rinse from wells and add 0.5 mL of diluted mouse anti-hexon antibody to each well.

4.11. Incubate for 1 hour at 37°C.
4.12. Aspirate mouse anti-hexon antibody and gently rinse wells three times with 1 mL PBS/BSA.

4.13. Dilute rat-anti mouse antibody (HRP conjugate) 1:500 in PBS/BSA.

4.14. Aspirate final rinse from wells and add 0.5 mL of diluted rat anti-mouse (HRP conjugate) to each well.

4.15. Incubate for 1 hour at 37ºC.

4.16. Dilute 10X DAB substrate 1:10 with 1X Stable Peroxidase Buffer. Prepare enough for 0.5 mL/well.

4.17. Allow 1X DAB solution to come to room temperature.

Note: Do not allow the 10X DAB substrate to come to room temperature.

4.18. Aspirate Rat Anti-Mouse (HRP conjugate) and gently rinse each well three times with 1 mL PBS/BSA.

Color Development

4.19. Aspirate final rinse from wells and add 0.5 mL 1X DAB solution to each well.

4.20. Incubate at room temperature for 10 minutes.

4.21. Aspirate 1X DAB solution and add 1 mL PBS to each well.

Quantification and Calculation

4.22. Count a minimum of three fields of brown/black cells using a microscope with a 20X objective.

4.23. Calculate infectious units (ifu)/mL for each well using the following equation:

\[
\text{(brown cells/field)} \times \text{(fields/well)} \times \frac{1 \text{ mL}}{\text{volumes of virus [mL]}} \times \text{(dilution factor)}
\]

5. Data Collection and Management

5.1.1. All data will be collected on the attached data sheet and transferred to a laboratory notebook.

5.1.2. 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: _______________________

Fecha: _______________________

Director, VDL
VDL602.2 RAPID ASSAY FOR DETERMINING ADENOVIRAL VECTOR TITER

Director, QA/QC

Reviewed by: __________________________
Director, Vector Production

Date Issued: 1/30/2006    Replaces VDL 602.0

Annual Review:

2011

Reviewed without changes                Changed and this version archived
Reviewed by: __________________________
QA/QC by: __________________________
Date: 7/8/2011    Replaces VDL 602.1

2012

Reviewed without changes                Changed and this version archived
Reviewed by: __________________________
QA/QC by: __________________________
Date: __________________________

2013

Reviewed without changes                Changed and this version archived
Reviewed by: __________________________
QA/QC by: __________________________
Date: __________________________
VDL602.2 RAPID ASSAY FOR DETERMINING ADENOVIRAL VECTOR TITER

Rapid Titer

Virus Name:__________________________________________

Titer Date:__________________________________________

Final Titer (pfu/ml):__________________________________

Assay conducted by:__________________________________

Results reviewed by:_______________________________

10^-6  ____________  10^-6  ____________

10^-7  ____________  10^-7  ____________

10^-8  ____________  10^-8  ____________