

#### CENTER FOR CELL & GENE THERAPY VECTOR DEVELOPMENT LABORATORY, 10<sup>TH</sup> FLOOR, ALKEK BUILDING ONE BAYLOR PLAZA, HOUSTON, TEXAS 77030



# VDL601.3 STANDARD ASSAY FOR DETECTION OF REPLICATION COMPETENT ADENOVIRUS

### 1. Purpose

- 1.1. The purpose of this protocol is to detect replication competent adenovirus (RCA) in a purified adenoviral vector.
- 1.2. In this assay, the sample is tested for its ability to infect A549 cells through a round of cell cuture infection.
- 1.3. 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 **Biosafety Level 2** 2.5. GLP Good Laboratory Practice 2.6. RCA **Replication Competent Adenovirus** 2.7. Antibiotics Penicillin/Streptomycin/anti-mycotic 2.8. FBS Fetal Bovine Serum 2.9. DMEM Dulbecco's Modified Eagle's Medium and 1% antibiotic 2.10. DMEM with 2% FBS DMEM2 2.11. DMEM10 DMEM with 10% FBS 2.12. DMEM20 DMEM with 20% FBS and 2% antibiotics 2.13. Trypsin 0.25% Trypsin-EDTA 2.14. PBS Phosphate Buffer Saline 2.15. Ad5 Wild-type Adenovirus subtype 5 2.16. PC Positive Control 2.17. NC **Negative Control** 

### 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

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- 3.1.2. 37°C/5% CO<sub>2</sub> incubator
- 3.1.3. Pipette aid
- 3.1.4. Inverted microscope with 20X objective
- 3.1.5. Vortex
- 3.1.6. Table top centrifuge

#### 3.2. Materials

	3.2.1.	50 mL conical tube	Corning
	3.2.2.	15 mL conical tube	Corning
	3.2.3.	150 mm cell culture dishes	Corning
	3.2.4. Serological pipets		Corning
	3.2.5.	Sterile pipet tips	VWR
	3.2.6.	1.7 ml tubes	Axygen
3.3	3.3. Reagents		
	3.3.1.	Trypsin	Cellgro
	3.3.2.	PBS	Invitrogen
	3.3.3.	DMEM	Cellgro
	3.3.4.	DMEM2	VDL
	3.3.5.	DMEM10	VDL
	3.3.6.	DMEM20	VDL
	3.3.7.	FBS	Atlas
	3.3.8.	Antibiotics	Cellgro

### 3.4. Starting Materials

- 3.4.1. Purified adenoviral vector, 5x10<sup>12</sup> particle/mL.
- 3.4.2. Wild-type adenovirus subtype 5,  $1 \times 10^{12}$  part/mL.
- 3.4.3. 16, 150 mm dishes of 80% confluent A549 cells (See SOP VDL705.2 Growth and Maintenance of A549 cells).

### 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

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Label dishes of A549 cells as follows:

- PC-1
- PC-2
- PC-3
- NC-1
- Test-1~10 (in 10 testing dishes)
- IF-1,2 (in duplicate)

## Prepare dilutions of positive control (Ad5).

- 4.1. Make a 1:10,000 dilution of Ad5  $(1x10^{12} \text{ part/mL})$  in DMEM2. Label "dilution A". (50ml DMEM2 + 5uL wildtype  $[1x10^{12} \text{ particle/ml}]$ ).
- 4.2. Make a 1:1000 dilution of dilution A prepared in step 4.1 by placing 10  $\mu$ L of the dilution A into 10 mL of DMEM2. The resulting concentration of Ad5 should be 1x10<sup>2</sup> part/ $\mu$ L. Label "dilution B".

## Prepare dilution of test sample.

- 4.3. Place 10 μL of the test adenoviral vector into 990 uL of DMEM2. Infect cells
- 4.4. Change medium on cells to 10ml DMEM2.
- 4.5. To the dish labeled PC-1, add 2  $\mu$ L of dilution B.
- 4.6. To the dish labeled PC-2, add 20  $\mu$ L of dilution B.
- 4.7. To the dish labeled PC-3, add  $200\mu$ L of dilution B.
- 4.8. To the dish labeled NC-1, add 200  $\mu L$  PBS.
- 4.9. To each dish labeled Test-1~10, add 60  $\mu$ L of diluted test adenoviral vector.
- 4.10. To the dish labeled IF-1, 2, add 60  $\mu$ L of diluted test adenoviral vector plus 20 $\mu$ L of "dilution B".

Note: Two dishes will be infected in this manner to produce duplicate tests.

- 4.11. Return dishes to the 37°C/5% CO<sub>2</sub> incubator for overnight incubation.
- 4.12. On the next day, bring the FBS concentration up to 10% by adding 10 mL DMEM20 to each dish.
- 4.13. Return dishes to the  $37^{\circ}C/5\%$  CO<sub>2</sub> incubator.
- 4.14. Every three or four days later, add 5 mL of DMEM10 to each dish, check the plates for streaks/comets.
- 4.15. Return dishes to the  $37^{\circ}C/5\%$  CO<sub>2</sub> incubator.
- 4.16. At the 11<sup>th</sup> day, check the plates for streaks/comets and record the result.





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### 5. Data Collection and Management

- 5.1.1. All data will be collected on the attached form and transferred to the 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:

Director, QA/QC

Reviewed by:

Director, Vector Production

Date Issued: 2/1/07 Replaces VDL 601.1

#### **Annual Review:**

2011

	Reviewed without changes	Changed and this version archived
Reviewed by:		
QA/QC by:		
Date Issued: 6/8	2011 Replaces VDL 601.2	
2012		
	Reviewed without changes	Changed and this version archived
Reviewed by:		
QA/QC by:		
Date:		

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2013

Reviewed without changes

Changed and this version archived

Reviewed by:

QA/QC by:

Date:



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## VDL601.3 STANDARD ASSAY FOR DETECTION OF REPLICATION COMPETENT ADENOVIRUS

RCA					
(Replication Competent Adenovirus)					
(3x10e10vp)					

Virus	Name:	

Assay Date:\_\_\_\_\_

The result at Day 11: Negative \_\_\_\_\_ Positive \_\_\_\_\_ in the Dish of Virus Only

Technician Initials:\_\_\_\_\_

Reviewed by \_\_\_\_\_

**Record of Tested Dishes** 

T1	Т6	
T2	T7	
T3	T8	
T4	Т9	
T5	T10	

#### Record of Tested IF Dishes

IF 1		IF2	
-			

#### Record of Control Dishes

Neg.		P2	
P1		P3	