VDL105.2 PREPARATION OF ADENOVIRAL VECTOR LYSAT

1. Purpose

1.1. The purpose of this protocol is to amplify an adenoviral vector from a crude virus stock for large-scale expansion.

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.	GLP	Good Laboratory Practice
2.4.	BSC	Biological Safety Cabinet
2.5.	CPE	Cytopathic Effect
2.6.	Antibiotics	Penicillin/Streptomycin/anti-mycotic
2.7.	FBS	Fetal Bovine Serum
2.8.	DMEM	Dulbecco's Modified Eagle's Medium with 1% Antibiotics
2.9.	DMEM10	DMEM with 10% FBS
2.10.	EDTA	ethylenediaminetetraacetic acid
2.11.	Trypsin	0.25% Trypsin-EDTA
2.12.	PBS	Phosphate Buffer Saline
2.13.	Tris	50mM Tris, pH 8.0

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. 37ºC/5% CO_2 incubator

3.1.3. Pipette aid

3.1.4. Inverted microscope with 20X objective

3.1.5. Table top centrifuge

3.1.6. Water bath set at 37 ºC

3.1.7. Microcentrifuge

3.2. Materials

3.2.1. 15 mL conical tubes — Corning

3.2.2.	150 mm cell culture dishes	Corning
3.2.3.	Serological pipets	Corning
3.2.4.	Sterile pipet tips	VWR
3.2.5.	250 mL centrifuge bottle	Sorvall
3.2.6.	100 mL glass bottle	Corning

3.3. **Reagents**

3.3.1.	Trypsin	Cellgro
3.3.2.	PBS	Invitrogen
3.3.3.	DMEM	Invitrogen
3.3.4.	FBS	Atlas
3.3.5.	Antibiotics	Cellgro

3.4. **Starting Materials**

3.4.1. 400µL crude virus (from step 4.22.1 in SOP: Plaque Expansion and Harvesting Adenoviral Vector).

3.4.2. Five 150 mm dish of 80% confluent 293 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.

3.5.3. A barcoded label that also contains virus name will be applied to all tubes, dishes, and Cell Stack used in the production of the virus.

4. Procedure

Infection of 293 cells in five 150 mm dishes with adenoviral vector

4.1. Remove sample from -80°C.

4.2. Subject the cell suspension to three freeze/thaw cycles. This involves thawing the cell suspension by placing the tube it is contained in, in a 37°C water bath. When it is completely thawed the tube is transferred to a dry ice/ethanol bath to freeze the cell suspension. The initial placing of the cell suspension in the -80°C freezer for storage is considered the first freeze.

NOTE: As the total volume is 400 µL both the freeze and the thaw cycles should be short, less than 5min.

4.3. Pellet cellular debris by centrifugation at 400 x g for 5 minutes.

4.4. Transfer the cleared viral lysate to a sterile 100 mL bottle and Q.S. to 100 mL with DMEM2 and mix well by pipetting.

4.5. Remove media from the 5, 150 mm dishes containing 80-90% confluent 293 cells.

4.6. Add 20 mL DMEM2/adenoviral vector mixture to each of the dishes.

4.7. Place the 150 mm dishes in the 37°C/5% CO_2 incubator.

4.8. On the next day, add 0.6 mL FBS to each plate.

NOTE: Microscopically inspect the cells every day in order to determine the degree of CPE. When 95-100% CPE is observed continue with the procedure. It will take 2-3 days before this stage of CPE is reached.

Note: If it takes longer than 4 days to reach 80-90% CPE, collect the viral lysate as described below in section 4.9-4.12, remove 1/5th of the volume (1mL) and repeat infection beginning with step 4.2.

Harvesting of adenoviral vector from five 150mm dishes of infected 293 cells

4.9. When 95-100% CPE is observed (all cells should be detached), transfer cell suspension to a 250 mL centrifuge bottle. Wash each dish with 10 mL PBS to detach residual cells.

4.10. Transfer wash to centrifuge bottle.

4.11. Pellet cells by centrifugation at 1,500 x g for 20 minutes.

4.12. Remove supernatant and resuspend the cell pellet in 5 mL of Tris. Pipette to resuspend cells to obtain a single-cell suspension.

4.13. Transfer the cell suspension to a 15 mL conical tube and freeze at -80°C until use.

5. Review and Revisions

Written by: _____
Director, VDL

Reviewed by: _____
Director, Vector Production

Reviewed by: _____
Director, QA/QC

Date Issued: 1/30/2006 Replaces VDL 105.0

Annual Review:

2011

Reviewed without changes Changed and this version archived

Reviewed by: _____ _____

QA/QC by: _____

Date Issued: 6 /15 /2011 Replaces VDL 105.1

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: _____

Alan R. Davis, Nelson A. Wivel, Joseph L. Palladino, Luan Tao, and James M. Wilson. Construction of Adenoviral Vectors, *Methods in Molecular Biology, Vol. 135: Developmental Biology Protocols, Vol. I,* Edited by: R.S. Tuan and C.W. Lo, Humana Press Inc., Totowa, NJ.