Title: Spectrophotometric Viral Particle Concentration Determination of Type 5 Adenovirus

SOP Number: 22102 **Revision Number: 05**

Supersedes: Revision 04 **Effective Date:**

Originator/Date: [redacted]

Approval/Date:

Approval/Date:

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1.0 Purpose

This procedure describes the method for measuring the concentration of adenoviral particles in purified preparation of virus using UV spectrophotometry at 260 nm.

2.0 Scope

The method described uses a spectrophotometer to determine the concentration of viral particles in a sample. Absorbance at 260 nm is measured in the presence of an ionic detergent. Particle concentration is proportional to the A_{260} value in the presence of detergent.

This procedure is made available through federal funds from theNational Cancer Institute. NIH, under contract [redacted]

The theoretical composition of Adenovirus 5 WT is 87% protein and 13% DNA. The concentration of adenoviral particles may be measured directly by UV-spectroscopy, since proteins have a UV absorbance at 277 nm due to their tryptophan and tyrosine content and double-stranded linear DNA has an absorbance maximum at 260 nm. In this assay, 0.1% SDS (w/v) disassembles the virus capsid into its component proteins and DNA. The absorbance of the lysed virus in SDS is measured at 260 nm. The viral particle concentration is calculated using a method described by Maizel, *et al*. In this method an absorbance of 1.00 AU (1-cm pathlength) at 260 nm corresponds to a concentration of 1.1×10^{12} viral particles/mL. This SOP is to be performed by trained Process Analytics personnel.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics (PA) has the authority to define this procedure.

3.2 PA personnel are responsible for the implementation of this procedure.

3.3 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.4 PA is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Safety Considerations

4.1 Adenovirus samples must be handled as biohazardous, BL-2. Wear gloves, sleeve covers, a second pair of gloves, safety glasses, and laboratory coat. Properly dispose of waste, including cuvette wash solutions according to **SOP 22923, *Procedures for Safe Handling and Decontamination of Viruses by BDP/BPA and Related Personnel***.

4.2 All work is to be performed in a biosafety cabinet (laminar flow hood), suitable for BL-2 containment.

4.3 Viral work should be scheduled during low traffic hours. Only personnel properly trained to work with virus should be present. Signs are to be posted on all doors entering the laboratory stating that active viral work is in progress and only authorized personnel are permitted entry.

5.0 Materials and Supplies

5.1 Milli-Q H_2O, Direct-Q H2O or approved BDP equivalent.

5.2 Parafilm, BDP PN 20465, or approved BDP equivalent.

5.3 Gloves, BDP PN 20457, or approved BDP equivalent.

5.4 Sleeve covers, BDP PN 20120, or approved BDP equivalent.

5.5 Reinforced Wipes, BDP PN 20354, or approved BDP equivalent.

5.6 Sterile pipets, 5 mL, BDP PN 20104, or approved BDP equivalent.

5.7 Sterile pipets, 10 mL, BDP PN 20100, or approved BDP equivalent.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

5.8 Pipet tips, 10 µL Aerosol Barrier, BDP PN 20335, or approved BDP equivalent.

5.9 Pipet tips, 1000 µL Aerosol Barrier, BDP PN 20769, or approved BDP equivalent.

5.10 Nalgene Media Bottles – 1 L, BDP PN 20160, and 125 mL, BDP PN 20159, or approved BDP equivalent.

5.11 Adenovirus Reference Standard, Wildtype Adenovirus 5 Std., 2 x 10^{11}/vial, BDP PN 30363, or approved BDP equivalent.

5.12 GST Buffer: 20 mM Tris-HCl, 25 mM NaCl, 2.5% Glycerol (w/v), pH 8.0. Store at 2 - 30ºC. Buffer is stable for 12 months.

5.12.1 GST Buffer prepared as:

5.12.1.1 20 mL 1M Tris-HCl pH 8.0, BDP PN 10097, or BDP approved equivalent.

5.12.1.2 5.0 mL 5M NaCl, BDP PN 10044, or approved BDP equivalent.

5.12.1.3 19.8 mL Glycerol, BDP PN 10125, or approved BDP equivalent.

5.12.1.4 Bring volume to 1.0 L with Milli-Q H_2O, Direct-Q H_2O or approved BDP equivalent.

Note: All Buffers and Solutions should be recorded in the PA Solutions Logbook per **SOP 22702, *Solutions Used in Process Analytics***.

5.13 10% Sodium dodecyl sulfate (SDS), BDP PN 30532, or approved BDP equivalent.

5.14 Bleach, BDP PN 30136.

5.15 Cavicide, BDP PN 10168.

5.16 Dispatch, BDP PN 10167.

5.17 Septihol, BDP PN 30129.

5.18 Methanol, BDP PN 10115, or BDP approved equivalent.

5.19 Cuvette Cleaning Solution, or equivalent solution prepared as:

5.19.1 2 mL Hellmanex II®, BDP PN 30785 or approved BDP equivalent.

5.19.2 Bring volume to 100 mL with Milli-Q H2O, Direct-Q H2O or approved BDP equivalent.

5.20 Microcentrifuge tubes, 1.5 mL capacity, BDP PN 20595 or approved BDP equivalent.

5.21 Biohazard pipet keeper BDP PN 21338.

5.22 Biohazard benchtop keeper BDP PN 21491.

5.23 Autoclave bags size 24 x 36 BDP PN 20728.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

5.24 Calibrated pipettors: 1-20 µL, 20-200 µL, and 200-1000 µL.

5.25 Beckman DU 640i Spectrophotometer, Beckman Coulter DU 800 Spectrophotometer or approved BDP equivalent.

5.26 Matched quartz cuvettes, 1-cm path length.

5.27 Aspirator cuvette cleaner with vacuum line.

5.28 Potassium Dichromate in Perchloric Acid U/V and Visual Spectrophotometry Standards, BDP MEF 78170 or approved BDP equivalent.

6.0 Procedure

6.1 Turn on the power to the spectrophotometer. Turn on UV and Visible lamps. Refer to **SOP 22158, *Operation of the Beckman DU Series 600 Spectrophotometer*** or **SOP 22941**, ***Operation of the Beckman Coulter DU 800 Spectrophotometer***.

6.2 Allow instrument to warm up (approximately one hour).

6.3 Biological Safety Cabinet Preparation

6.3.1 Prior to working with adenovirus prepare the biosafety cabinet as follows:

- Decontaminate the biosafety cabinet with Cavacide or Dispatch followed by cleaning with Septihol. Record usage in appropriate logbook.
- Place a Nalgene Media Bottle containing 100-200 mL of 100% Bleach in the biosafety cabinet.
- Saturate a reinforced wipe with Cavicide or Dispatch and place in the biosafety cabinet.
- Place sufficient pipet tips and 1.5 mL microcentrifuge tubes for use in sample preparation in the biosafety cabinet.
- Place Matched quartz cuvettes in the biosafety cabinet.
- Place a biohazardous benchtop keeper and biohazardous pipet keeper in the biosafety cabinet.

6.4 System Suitability

6.4.1 At the Main Screen of the Spectrophotometer, select Fixed Wavelength. Select Method $\mathbf{A_{257}}$.

6.4.2 Record the MEF number and calibration due date of the Potassium Dichromate UV/Vis Standards on page 3 of Form 22102-01.

6.4.3 Potassium Dichromate UV/Vis Standards are calibrated annually. To locate the current 3-Sigma range values for 257 nm, go to H:\BDP_Public\PA Files\Molecular Biology\Reports\Assay Development\Absorbance Measurements\BDP Absorbance Standards. Open the file for the Potassium Dichromate UV/Vis Standards currently being used as indicated by the MEF number (see 6.4.2). Print the current 3-Sigma range values for 257 nm and include the print out with the experimental data. Enter the values on page 3 of Form 22102-01.

6.4.4 Place the Potassium Dichromate Blank in sample compartment number 1 (furthest from the operator)

6.4.5 Close the lid before performing analysis.

6.4.6 Left click the Blank icon to blank the spectrophotometer.

6.4.7 Left click on the sample number. Label the sample “Blank”.

6.4.8 Read the absorbance $\mathbf{A_{257}}$ of each Potassium Dichromate Standard (0, 20, 40, 60, 80, and 100). Print results and record each absorbance obtained on page 3 of Form 22102-01.

6.4.9 Verify that each reading obtained is within the acceptable range and circle the appropriate response (Pass/Fail) on Form 22102-01. Each of the standards must pass in order to proceed with the Adenovirus sample. If a failure occurs repeat steps 6.4.1 through 6.4.9. If after a 2nd attempt one or more of the standards fail notify the PA supervisor. Do not proceed to next step of procedure until system suitability has been successfully performed.

6.5 Sample Analysis

Note: All sample preparation should be performed in a BL-2 biosafety cabinet. All pipeting should be done using pipetors designated for viral use. Used, sterile pipets should be discarded in a biohazard pipet keeper and pipet tips should be discarded in a biohazard benchtop keeper.

6.5.1 Prepare a buffer blank. Add 495 µL GST Buffer and 5 µL of 10% SDS solution to a 1.5 mL microcentrifuge tube. Triturate 5-10 times to mix. Transfer buffer blank to a quartz cuvette, cap it and secure the cap with parafilm (make sure the reading path of the cuvette is not obscured). Record dilutions, buffers and pipets used on Form 22102-01.

6.5.2 Decontaminate the outside of the cuvette using a reinforced wipe saturated with Cavacide or Dispatch. Wipe the outside of the cuvette using a reinforced wipe saturated with Septihol.

6.5.3 At the Main Screen of the Spectrophotometer, select Fixed Wavelength. Select Method $\mathbf{A_{260}}$.

6.5.4 Place the buffer blank (GST/SDS buffer) cuvette in sample compartment number 1 (furthest from the operator) with the transparent side of the cuvette in the field of the light path (left to right).

6.5.5 Close the lid before performing analysis.

6.5.6 Left click **Blank**.

6.5.7 Left click on the sample number to label the buffer blank.

6.5.8 Right click to read, or click "**Read Samples**" at the top left of the screen if using Beckman DU 640i. If using Beckman Coulter DU 800, left click "**Go Read**" Icon. The GST buffer/SDS solution should now have an absorbance at 260 nm of 0.00 au.

6.5.9 Thaw frozen samples at room temperature in a biosafety cabinet. Wipe outside of sample container with a reinforced wipe saturated with Cavicide or Dispatch. Mix thoroughly after the thaw, by trituration (pipetting the solution up and down several times) using a sterile pipette. Triturate 5-10 times to mix.

6.5.10 If the test article is not sampled within 30 minutes it should be stored on wet ice or at 2-8°C until use. Immediately prior to use, the sample should be warmed to room temperature. Do not use a hot water bath to warm the sample.

6.5.11 Adenovirus samples should be diluted to a concentration of approximately 1.1 x 10^{11} to 5.5 x 10^{11} vp/mL in GST buffer. This range ensures that the reading frame of the spectrophotometer is between 0.1 and 0.5 absorbance units. For samples with an unknown viral concentration several dilutions may be required to ensure an accurate reading is obtained. A 1:2 dilution is typically used as a starting point for analysis. If the initial dilution yields a result outside of the linear range of the instrument perform additional dilutions as needed. Record dilutions, buffers and pipets used on Form 22102-01.

6.5.12 Working in the Biosafety cabinet, dilute the Adenovirus Reference Standard (Wildtype Adenovirus 5 Std., 2 x 10^{11}/vial, BDP PN 30363, or BDP approved equivalent) 1:2 with GST buffer. Add 495 µL of diluted reference standard and 5 µL of 10% SDS solution to a 1.5 mL microcentrifuge tube. Triturate 5-10 times to mix. Incubate for 5 minutes at room temperature in the BSC. Transfer solution to a quartz cuvette, cap it and secure the cap with parafilm (make sure the reading path of the cuvette is not obscured).

6.5.13 Decontaminate the outside of the cuvette using a reinforced wipe saturated with Cavacide or Dispatch. Wipe the outside of the cuvette using a reinforced wipe saturated with Septihol.

6.5.14 Place the Adenovirus Reference Standard cuvette in the spectrophotometer and read the absorbance at 260 nm three times. Record each reading and the calculated mean on Form 22102-01.

6.5.15 Working in the Biosafety cabinet, add 495 µL of appropriately diluted Adenovirus sample and 5 µL of 10% SDS solution to a 1.5 mL microcentrifuge tube. Triturate 5-10 times to mix. Incubate for 5 minutes at room temperature in the BSC. Transfer solution to a quartz cuvette, cap it and secure the cap with parafilm (make sure the reading path of the cuvette is not obscured).

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

6.5.16 Decontaminate the outside of the cuvette using a reinforced wipe saturated with Cavacide or Dispatch. Wipe the outside of the cuvette using a reinforced wipe saturated with Septihol.

6.5.17 Place the Adenovirus sample cuvette in the spectrophotometer and read the absorbance at 260nm three times. Record each reading and the calculated mean on Form 22102-01.

6.5.18 Repeat steps 6.5.15 through 6.5.17 for each additional sample.

6.6 Cuvette Decontamination

6.6.1 Return cuvettes to biosafety cabinet.

6.6.2 Remove parafilm and discard in biohazard benchtop keeper.

6.6.3 Using a pipet, transfer sample into the Nalgene media bottle containing 100% bleach. Incubate at room temperature for at least 30 minutes.

6.6.4 Rinse cuvette with Milli-Q H_2O, Direct-Q H_2O or approved BDP equivalent.

6.6.5 Wipe outside of cuvettes using a reinforced wipe saturated with Cavicide or Dispatch. Wipe the outside of cuvettes using a reinforced wipe saturated with Septihol. Cuvettes can now be safely transported to the laboratory bench top for cleaning.

6.6.6 Decontaminate and remove all material from the biosafety cabinet. Decontaminate the biosafety cabinet with Cavacide or Dispatch followed by cleaning with Septihol. Record usage in appropriate logbook.

6.7 Cuvette Cleaning

6.7.1 Using the aspirator cuvette cleaner attached to a vacuum line, rinse cuvette with Milli-Q H_2O, Direct-Q H_2O or approved BDP equivalent.

6.7.2 Rinse cuvette with Cuvette Cleaning Solution.

6.7.3 Rinse with Milli-Q H_2O, Direct-Q H_2O or approved BDP equivalent until the foaming stops.

6.7.4 Rinse with Methanol.

6.7.5 Allow the cuvette to remain on vacuum until the cuvette appears dry.

7.0 Documentation

7.1 Save results using the PA Test Request number as the file name.

7.2 Record spectrophotometer usage in the UV/VIS User Logbook.

8.0 Determination of Results

8.1 Print results by clicking on the print button in the upper right hand corner of the screen.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

8.2 In order to calculate the Concentration of Viral Particles (VP) in the sample, multiply the mean absorbance from the printout by 1.1×10^{12}. Correct for dilution factors if any. The resulting number is the VP calculation for the sample. Record results on Form 22102-01.

8.3 Attach results to PA Request Form 22002-01.

9.0 References and Related Documents

9.1 **SOP 22158** *Operation of the Beckman DU Series 600 Spectrophotometer*

9.2 **SOP 22702** *Solutions Used in Process Analytics*

9.3 **SOP 22923** *Procedures for Safe Handling and Decontamination of Viruses by BDP/BPA and Related Personnel*

9.4 **SOP 22941** *Operation of the Beckman Coulter DU 800 Spectrophotometer*

9.5 Maizel, J.V., White, D.O., Scharff, M.D., "The Polypeptides of Adenovirus," (1968) Virology 36: 115-125.

9.6 http://www.wilbio.com/ReferenceMaterialProjects/pdfs/CharacterizationSopWorksheets001.pdf

10.0 Attachments

10.1 **Attachment 1** Form 22102-01, Adenovirus Viral Particle Concentration Determination Worksheet

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

Attachment 1

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Adenovirus Viral Particle Concentration Determination Worksheet

PA Number: _____ **Analyst:** _____ **Date:** _____

UV/Vis Spectrophotometer MEF #: _____ **Calibration Due Date:** _____

Pipet(s) #: _____

Sample Dilutions:

GST/SDS Buffer Blank (495 µL GST Buffer/ 5 µL 10% SDS):
GST Buffer Lot# _____ Exp. Date:_____
10% SDS Lot#_____ Exp. Date:_____

Test Sample #1

Name: _____ Lot #_____
Buffer:_____ Lot# _____ Exp. Date:_____
Dilution: ____Volume of Diluent: ___µL Volume of Test Sample: ___µL Volume of 10% SDS: ___ µL
1st Reading (A260 nm): _____au
2nd Reading (A260 nm): _____au
3rd Reading (A260 nm): _____au
Mean Result: _____au

Calculation: _____ (Mean Result) x _____ (Dilution Factor) x 1.1×10^{12} vp/mL = _____ vp/mL (Viral Concentration)

Test Sample #2

Name: _____ Lot #_____
Buffer:_____ Lot# _____ Exp. Date:_____
Dilution: ____Volume of Diluent: ___µL Volume of Test Sample: ___µL Volume of 10% SDS: _____µL
1st Reading (A260 nm): _____au
2nd Reading (A260 nm): _____au
3rd Reading (A260 nm): _____au
Mean Result: _____au

Calculation: _____ (Mean Result) x _____ (Dilution Factor) x 1.1×10^{12} vp/mL = _____ vp/mL (Viral Concentration)

Viral Concentration Calculation: Virus particles per mL = 1.1×10^{12} vp/mL x Dilution Factor x OD_{260} Mean Result

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].

Attachment 1 (Continued)

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Adenovirus Viral Particle Concentration Determination Worksheet

Test Sample #3

Name: _____ Lot #_____
Buffer:_____ Lot# _____ Exp. Date:_____
Dilution: ____Volume of Diluent: ____µL Volume of Test Sample: ___µL Volume of 10% SDS: ____µL
1st Reading (A260 nm): _____au
2nd Reading (A260 nm): _____au
3rd Reading (A260 nm): _____au
Mean Result: _____au

Calculation: $\frac{\text{Mean Result}}{\text{Mean Result}}$ x $\frac{\text{Dilution Factor}}{\text{Dilution Factor}}$ x 1.1×10^{12} vp/mL = $\frac{\text{Viral Concentration}}{\text{Viral Concentration}}$ vp/mL

Test Sample #4

Name: _____ Lot #_____
Buffer:_____ Lot# _____ Exp. Date:_____
Dilution: ____Volume of Diluent: ___µL Volume of Test Sample: ___µL Volume of 10% SDS: _____µL
1st Reading (A260 nm): _____au
2nd Reading (A260 nm): _____au
3rd Reading (A260 nm): _____au
Mean Result: _____au

Calculation: $\frac{\text{Mean Result}}{\text{Mean Result}}$ x $\frac{\text{Dilution Factor}}{\text{Dilution Factor}}$ x 1.1×10^{12} vp/mL = $\frac{\text{Viral Concentration}}{\text{Viral Concentration}}$ vp/mL

Reference Standard

Name: _____ Lot #_____
Buffer:_____ Lot# _____ Exp. Date:_____
Dilution: ____Volume of Diluent: ___µL Volume of Test Sample: ___µL Volume of 10% SDS: ___µL
1st Reading (A260 nm): _____au
2nd Reading (A260 nm): _____au
3rd Reading (A260 nm): _____au
Mean Result: _____au

Calculation: $\frac{\text{Mean Result}}{\text{Mean Result}}$ x $\frac{\text{Dilution Factor}}{\text{Dilution Factor}}$ x 1.1×10^{12} vp/mL = $\frac{\text{Viral Concentration}}{\text{Viral Concentration}}$ vp/mL

Viral Concentration Calculation: Virus particles per mL = 1.1×10^{12} vp/mL x Dilution Factor x OD_{260} Mean Result

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Attachment 1 (Continued)

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PA Number:_____ **Analyst:**_____ **Date:**_____

Potassium Dichromate UV and Visual Spectrophotometry Standards

MEF_____ Calibration Due Date:_____

Potassium Dichromate UV/Vis Standards					
Conc. (mg/L)	A_{257} Low	Actual Reading	A_{257} High	Within Range	
0				Pass	Fail
20				Pass	Fail
40				Pass	Fail
60				Pass	Fail
80				Pass	Fail
100				Pass	Fail

Performed by: _____ Date: _____

Reviewed by: _____ Date: _____

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract [redacted].