DETERMINATION OF ADENOVIRUS PARTICLE COUNT

BY UV SPECTROPHOTOMETRY

1. PURPOSE

This Standard Operating Procedure describes the method for measuring the concentration of viral particles using UV/Vis spectrophotometry at 260 and 280 nm.

2. SCOPE

This procedure is performed on GMP lots of adenovirus drug substance and drug product at the time of release for clinical use, as well as for ongoing stability studies. This method may also be used for R&D and in-process samples.

3. BACKGROUND

The theoretical composition of adenovirus ONYX-015 is 87% protein and 13% DNA. The concentration of viral 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 DNA has an absorbance maximum at 260 nm. Intact viral particles have a diameter between 65 to 85 nm. Their high organization and potential aggregation state cause light scattering which can bias the UV-measurement. In this assay, 0.1% SDS disassembles the virus into its component proteins and DNA. The UV-absorbance of the lysed virus in SDS is measured at 320 nm for the baseline and reference, at 260 nm for its DNA content and at 280 nm for its protein content. 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 1.1×10^{12} viral particles/mL.

4. REFERENCES

Operating Manual for Beckman Spectrophotometer, Model DU®640B

Maizel, J.V., White, D.O., Scharff, M.D., “The Polypeptides of Adenovirus”, Virology *36*, 115-125, 1968

5. RESPONSIBILITIES

Quality Control (QC) is responsible for performing this assay according to this procedure and reviewing data prior to submission to QA.

Quality Assurance (QA) is responsible for reviewing final data.

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6. EQUIPMENT AND MATERIALS (Alternate components may be substituted if equivalent)

Equipment
UV/VIS Spectrophotometer, DU® 640B, Beckman
Microcell Quartz Cuvette (10 mm path length, 100 µL), Beckman, Cat. #523452
Eppendorf tubes, 1.5 mL

Materials
Sodium Dodecyl Sulfate (SDS) solution, 20%, sterile, AMRESCO, Cat. #0837
Sodium Dodecyl Sulfate (SDS) powder, >99% purity, Ultrapure Bioreagent, J.T. Baker, Cat. #JT4095
Water, distilled, sterile, Gibco BRL, Cat. #15230-170
Appropriate formulation buffer, filtered, refrigerated

7. REAGENT PREPARATION

Note: Label all reagents with the reagent name, preparer's initials, preparation date, appropriate storage conditions, and expiration dates.

7.1 Formulation Buffer: Obtain or prepare sterile formulation buffer. Store at 2-8°C. Expires after 3 months.

7.2 Assay Reagent 1 (0.2% SDS in Formulation Buffer): Combine 100 mL Formulation Buffer with 0.2 g SDS. Alternatively, combine 1 mL of 20% SDS stock with 99 mL Formulation Buffer. Store at 2-8°C. Expires after 3 months.

7.3 Blank Buffer (0.1% SDS in Formulation Buffer): Combine equal volumes of Assay Reagent 1 and Formulation Buffer. Prepare at least 1 mL volume. Mix well prior to use. Prepare fresh on day of use.

7.4 Test samples: For samples with an expected concentration of 0.5-2 x 10^{12} particles/mL, combine equal volumes of sample and Assay Reagent 1 (e.g., 300 µL Assay Reagent 1 and 300 µL virus sample).

7.4.1 For concentrations expected to be > 2 x 10^{12} particles/mL, dilute sample with Formulation Buffer to approximately 1 x 10^{12} particles/mL. Combine equal volumes of diluted sample and Assay Reagent 1.

7.4.2 For unknown samples, absorbance reading will determine if dilution is appropriate. Refer to Step 12.2.

7.4.3 Each preparation should be at least 300 µL.

7.5 Repeat step 7.4 to make three independent preparations for each test sample.

7.6 Virus Reference Standard: Prepare one sample of a reference standard as in step 7.4.

8. PROCEDURE FOR SYSTEM SUITABILITY CHECK

8.1 Each assay is assigned a UV Particle Count number at this time. Assays are numbered sequentially from 001. The number is recorded on the UV Particle Count Data sheet (Form 1).

8.2 Turn on the power and allow the spectrophotometer to warm up for at least 30 minutes prior to use by pressing UV off and VIS off. This will turn on lamps.

8.3 Select Method: A:QCSOPOD (preprogrammed to 260/280 ratio).

8.4 Test for instrument noise and correct operation of UV-Lamp:
- With no cuvette in place, blank the instrument and press “read samples”.
- Attach print out of reading to test data sheet.

8.5 Test for a clean cuvette
- Clean cuvette thoroughly with water.
- Check for scratches, cracks and clean optical windows.
- Fill the cuvette with water and ensure that the sides of the cuvette are clean and dry. Wipe the optical windows and place the cuvette in the sample compartment.
- To measure the absorbance of the cuvette and water, press “read samples”.
- Zero the instrument (i.e. click “blank”) to set the water readings to “0”.
- Press “read samples” to read the absorbance after the water correction.
- Attach a printout of both readings to the test data sheet.

8.6 Test for correct background reference (blank buffer)

Note: **When using a micro-cuvette (100 µL), keep the cuvette in the cuvette holder for all subsequent measurements of blank, test, and reference samples.** Removing the micro-cuvette may invalidate the blank correction.

- Rinse the cuvette three times with Blank Buffer, then load 100 µL of Blank sample.
- To measure the absorbance of the Blank Buffer, press “read samples”.

8.7 Zero the instrument to set the Blank Buffer readings to “0”.

8.8 Analyze Blank Buffer to confirm that blank buffer correction was performed by pressing “read samples”. Print out and attach both readings of Blank Buffer to the test data sheet (Form 1).

8.9 Evaluate the system suitability per section 9.

9. SYSTEM SUITABILITY REQUIREMENTS

9.1 Instrument noise and correct operation of UV lamp.

- None of the absorbance readings should be > ± 0.005 AU
- If any of the readings are > ±0.005 AU, repeat step 8.4. If the UV-lamp is still not stable, check the UV-source. If the problem persists, notify your supervisor or arrange for equipment to be serviced.
- If the instrument is out of order, place an OUT OF ORDER tag on it. Sign and date the OUT OF ORDER tag, and explain why the instrument is out of order. Notify all QC staff about the out of order instrument.

9.2 Clean cuvette

- Cuvette should have no scratches or stains on the optical window.
- None of the absorbance readings should be > 0.25 AU.
- The difference between A_{260} and A_{320} should be ≤ 0.05 AU.
- If the above criteria are not met, use a different cuvette or clean the cuvette. Refer to Attachment 1 for a recommended cleaning procedure.
- Dispose of damaged cuvette into Sharps container.

9.3 Background reference (Blank Buffer)

- None of the absorbance readings should be > 0 ± 0.04 AU. If these criteria are not met, prepare a fresh buffer.

9.4 If system meets suitability requirements, proceed to section 10.

10. PROCEDURE FOR SAMPLE ANALYSIS

10.1 Analyze Reference Standard as follows:

10.1.1 Record lot # and expected concentration of reference standard on Form 1.

10.1.2 Rinse cuvette two times, once with formulation buffer, then with reference standard (100 µL/rinse). Then load 100 µL of reference standard to read.

10.1.3 Press “read samples” to measure absorbance of reference standard (reference standard is not read in triplicate).

10.1.4 Proceed with test sample analysis.

10.2 Rinse the cuvette two times, once with formulation buffer, then with test sample 1 (100 µL per rinse).

10.3 Fill cuvette with 100 µL of test sample 1, and measure the absorbance readings (press "read samples").

10.4 Repeat step 10.3 for the other two preparations of the same test sample (it is not necessary to wash the cuvette between readings of the same sample).

10.5 Repeat steps 10.2 through 10.4 for remaining test samples.

10.6 Record sample IDs and all raw sample absorbance measurements on Form 1 (A_{260}, A_{280}, A_{320}).

10.7 After analysis of the last sample, remove and clean the cuvette thoroughly with DI water. Turn the instrument off.

10.8 Attach a printout of the measurements to Form 1.

11. DATA ANALYSIS

11.1 Calculate the corrected A_{260} and A_{280} absorbance measurements and corrected ratio and record on Form 1.

Corrected $A_{260} = (A_{260} - A_{320})$
Corrected $A_{280} = (A_{280} - A_{320})$
Corrected ratio = (Corrected A_{260} / Corrected A_{280})
A_{260} = the absorbance at 260 nm
A_{280} = the absorbance at 280 nm
A_{320} = the absorbance used for background and scatter correction

11.2 Record the dilution factor and particles/mL on Form 1. Calculate the particle count according to Maizel et al. in particles/mL as follows:

Virus concentration [ptc/mL] = 1.1×10^{12} × corrected A_{260} × DF
Where:
1.1×10^{12} = the number of virus particles per mL per 1 AU at 260 nm
DF = sample dilution factor

11.3 Report the concentration of virus in particle/mL to three significant figures and the corrected absorbance ratio of 260 to 280 nm to three significant figures.

11.4 Calculate the average concentration (particles/mL), standard deviation (SD), % Relative Standard Deviation (RSD), and Average Ratio for each sample using a scientific calculator or appropriate standard software. All average calculations are reported to two significant figures.

11.5 Record all calculations on Form 1.

12. TEST ACCEPTANCE CRITERIA

12.1 Particle count for the reference standard sample should be within 10% of the expected value (refer to history file).

12.2 The absorbance reading at 260 nm should be between 0.1 and 1.0 AU. If necessary, repeat the measurements with a more or less concentrated sample. Record the new dilution factor on the test data sheet.

12.3 The absorbance reading at 320 nm should be ≤ 0.05. If > 0.05, interference from light scattering will be too high, and filtration may be necessary. Record all additional sample manipulation steps (filtration, centrifugation, etc.) on the test data sheet.

12.4 The particle count % RSD for three independent preparations of the same sample must be ≤ 5.0%

12.5 Average ratio of 260/280 = 1.2 – 1.4.

13. DOCUMENTATION

13.1 Submit the UV Particle Count Data (Form 1) to QC and subsequently to QA for review.

13.2 QC and QA will sign and date each form following review only when all data has been recorded correctly and completely, and any deviations have been documented as appropriate.

13.3 Retain completed and reviewed Data Sheets in the assay binder in QC for up to one year. After that time the original data are transferred to QC archives in QA.

13.4 A copy of assay results is made for each lot file, as appropriate.

Attachment 1
Recommended Procedure for Cuvette Cleaning

- Fill cuvette with Chromerge® for 1 hr or overnight, or heat for 10 sec in a microwave in a closed container (caution: read safety and handling sheet before using Chromerge®.)
- Rinse with at least 10 cuvette volumes of deionized water.
- Rinse three times with methanol.
- Allow to air dry or rinse methanol off with water.

Form 1
UV PARTICLE COUNT DATA SHEET
(Page 1 of 2)

UV Particle Count assay #:_______________

UV Spectrophotometer ID:__________________________________

Assay Reagent 1 Expiration Date:______________________

Reference Std. Lot # and expected concentration:____

Sample Preparation:____

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System Suitability

Sample ID	A 260 nm	A 280 nm	A 320 nm	Specification	Pass/Fail
No cuvette				0 ± 0.005	
Water				$\leq$ 0.25 **and** ($A_{260} - A_{320}$) = $\leq$ 0.05	
Water after re-zeroing				0 ± 0.005	
Blank Buffer				0 ± 0.04	
Blank Buffer after re-zeroing				0 ± 0.04	

Print-outs of system suitability attached: ❑

Comments:____

____

Performed by:____ Date: ____________________
QA/QC Review Stamp:

Form 1
UV PARTICLE COUNT DATA SHEET
(Page 2 of 2)

UV Particle Count assay #:_______________ Reference standard concentration is 90-110% of expected concentration: ❑ yes ❑ no

	Raw Data from print-out					Calculated Data								
Sample ID (P/N, L/N, Alias)	Vial #	Stability Time Pt.	A_{260}	A_{280}	A_{320}	Corrected A_{260}	Corrected A_{280}	Corrected Ratio 260/280	Final Dilution factor	Conc. (ptc/mL)	Avg. conc. ptc/mL	SD n=3	% RSD	Avg. Ratio
Ref. Sample														
Sample 1:														
Sample 2:														
Sample 3:														

Comments:____

Performed by: ____ Date: _______________ # pages attached:___________

QA/QC Review Stamp: