

**Transgene's Bid Submission to Participate in Characterization of Reference Material -Other Characterization  
RFP 10.0**

**Determination of adenovirus aggregation by photon correlation spectroscopy**

**1. Amount of Ad5 WT Reference Material required**

1.5 E10 particles

**2. Standard Operating Procedure**

See below

**3. Transgene experience of the method**

The light scattering method is routinely used to control the product at different stages of the purification process and to assess production lots formulated with different buffers during stability studies. A good correlation is observed between aggregation and loss of infectious titer. Moreover, aggregation rate can be linked to loss of virus particles after 0.2 micron filtration.

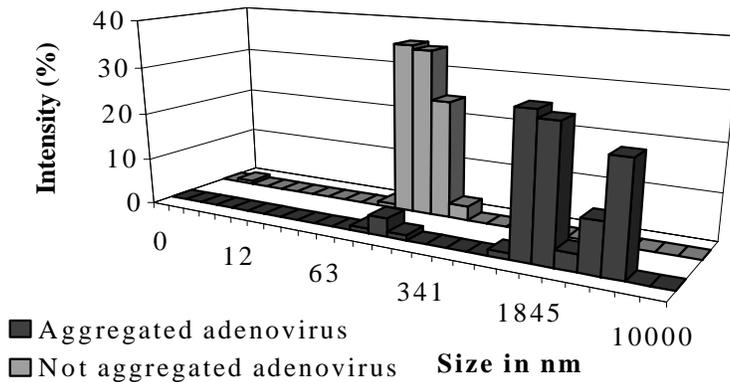
Full validation of the SOP according to ICH guidelines is in progress.

Example of an aggregated and a non aggregated product

Results obtained with an aggregated and a non aggregated adenovirus sample are listed in table 1 for unimodal size and polydispersity index and represented in figure 1 for SDP analysis.

**Table 1:** Typical unimodal size and polydispersity index for an aggregated and a not aggregated sample

Sample	Unimodal size in nm	Polydispersity index
Not aggregated batch	117.4	0.107
Aggregated batch	1174.5	1.482



**Figure 1:** Size distribution processor analysis (SDP intensity) of an aggregated and a not aggregated batch of adenovirus

## **4. Personnel qualifications, experience and training**

### **4.1 Personnel involved in performing the procedure**

Catherine FAHRNER, senior technician in Control Development and Quality Control

**General academic qualification:**

BTS (Brevet de technicien supérieur) in Biophysics. BTS is the equivalent to the high school baccalauréat + 2 additional years of study and examinations.

**Experience and training:**

Employed by Transgene since 1987, 7 years in Biochemistry Department and 7 years in Control Development and Quality Control.

**Qualified for:**

Determination of adenovirus aggregation by photon correlation spectroscopy.

### **4.2 Personnel involved in reviewing the data**

Edwige BONFILS, senior scientist, Head of the Control Development

**General academic qualification:**

Maîtrise in Biochemistry (roughly equivalent to a B.Sc.). D.E.A in Organic Chemistry (roughly equivalent to a M.Sc.). Ph.D.; in Biochemistry, subject: Targeting of antisense oligonucleotides *via* glycosylated carriers.

**Experience and training:**

Seven year experience in research, development and production (oligonucleotide chemistry, biochemistry and molecular biology) with Appligene. Employed by Transgene since September 1998.

**Qualified to review the data of:**

Determination adenovirus aggregation by photon correlation microscopy

## **5. Equipment**

### **3.1 Laser N4 Plus Submicron Particle Sizer (COULTER)**

Annual operating qualification performed by a trained and qualified technician of COULTER company

### **3.2 Pipettors (GILSON)**

3-monthly operating qualification

## **6. Delay to perform the procedure, and review and report results**

Two weeks after sample receipt.

**Adenoviral Reference Material Working Group  
Bid Submission Form  
Other characterization  
RFP 10.0**

**Please complete the following fields:**

*Contact Information – RFP 10.0*

*Contact Individual:	Daniel MALARME
Institution:	TRANSGENE
Address:	11, rue de Molsheim 67082 Strasbourg cedex FRANCE
Phone Number:	33 388 279215
Fax Number:	33 388 279141
Email Address:	malarme@transgene.fr

**\*If laboratories are submitting a proposal as a group, a main contact should be provided along with contact information for each participating laboratory (attach additional copies of this form).**

	<b>Standard operating procedure for the determination of adenovirus aggregation by photon correlation spectroscopy</b>	Reference: N/A
--	--	-------------------

## 1. OBJECTIVE

Determination of adenovirus aggregation by photon correlation spectroscopy.

## 2. PRINCIPLE

The viral particles undergoing a Brownian motion are detected and analyzed by illuminating the viral suspension with a laser and measuring the scattered light with a laser. Large, relatively slowly moving particles, change position slowly and cause slow intensity fluctuations at the detector; conversely small, quickly moving particles cause rapid intensity fluctuations. Photon Correlation Spectroscopy (PCS) sizes particles by characterizing the exact time scale of the random intensity fluctuations caused by the changing patterns of the diffusing Brownian particles.

Unimodal analysis determines the mean particle size and standard deviation of the size distribution. Size Distribution Processor analysis determines the particle size distribution. Aggregation is determined by comparison of Unimodal and SPD data for an adenovirus sample to those for an adenovirus reference diluted in the same buffer.

## 3. MATERIALS AND METHODS

### 3.1 Materials

0.5 ml micro-disposable cuvettes (Dustcher n° 30101 or equivalent)

Cuvette caps (Polylabo n° 52743 or equivalent)

Sterile microliter pipette tips, with aerosol filters for 1-20 µl, 20-200 µl, 200-1000 µl volumes

N4 Size Control L100 and L500 of the Colloï dal Particle Control Kit (Beckman Coulter n° 6602336)

### 3.2 Equipment

Biosafety cabinet (laminar flow hood) suitable for BL-2 containment

Adjustable microliter pipettes 1-20 µL; 20-200 µL; 200-1000µL

Submicron Particle Sizer Coulter N4 plus or equivalent

### 3.3 Reagents

Ultra-pure water (Milli-Q water or equivalent), filtered with 0.2 micron filter

Sample formulation buffer (10 ml), filtered with 0.2 micron filter

Adenovirus reference lot.

### 3.4 Method

#### 3.4.1 Sample preparation

##### 3.4.1.1 Dilution of the controls

Using 0.5-ml micro-disposable cuvettes, make a dilution of N4 Size Control L100 and L500 to obtain a scattering intensity at 90 degree within the limits specified in the instrument software.

Cap the cuvettes.

	<b>Standard operating procedure for the determination of adenovirus aggregation by photon correlation spectroscopy</b>	Reference: N/A
--	--	-------------------

#### 3.4.1.2 Dilution of the reference

Dilute the reference virus in the buffer of the sample to be tested.

Open the ampoule of reference and homogenize the suspension by gentle pipetting. Pipette 50 µL and add it to 300 µl of sample buffer in a 0.5 micro-disposable cuvette.

Cap the cuvette.

#### 3.4.1.3 Preparation of the test sample

Using 0.5-ml micro-disposable cuvettes, properly dilute the sample in its formulation buffer to obtain a scattering intensity at 90 degrees within the limits specified in the instrument software. If the particle concentration is known, realize a suspension at 5.0E10 particles/ml. Perform the dilutions in triplicate. Cap the cuvettes.

### 3.4.2 Measurement

Turn on the power to the Submicron Particle Sizer. Allow the instrument to warm up for the time recommended by the equipment manufacturer.

Invert the cuvettes containing the samples twice to disperse the suspensions uniformly. Leave them for two to three minutes at 20°C before measurement.

#### 3.4.2.2 Run

Check the cuvette for fingerprints and bubbles. Wipe it with a lint-free tissue before placing it into the sample compartment.

Place the sample in the sample compartment and select the angle at which the measurement will be performed.

Check the intensity, if the counts are too high or too low, perform another dilution to obtain a scattering intensity at 90 degrees within the limits specified in the instrument software.

Run the sample using a run profile based on the table below:

Parameter	Setting
Sample time	Automatic
Prescale	Automatic
Run time	3 minutes
Temperature	25°C
Equilibration time	3 minutes
Angle	90°
Analysis	Unimodal

Run the sample according to the following list:

- 1) N4 Size Control L100
- 2) N4 Size Control L500
- 3) Reference in the test sample formulation buffer
- 4) Test sample dilution 1
- 5) Test sample dilution 2
- 6) Test sample dilution 3

	<b>Standard operating procedure for the determination of adenovirus aggregation by photon correlation spectroscopy</b>	Reference: N/A
--	--	-------------------

## 4. RESULTS

### 4.1 Data processing

Record the data for intensity, polydispersity index (P.I.), unimodal size and SPD results on the appropriate form and print the bar chart and the table for the size distribution (SDP intensity).

For the test sample, calculate the mean values for intensity, polydispersity index (P.I. inferior to zero is consider as 0), unimodal size. Calculate RSD (in %) for unimodal size.

### 4.2 Results

Unimodal size of the reference diluted in the sample formulation buffer is considered as the expected size value for a not aggregated sample.

A sample is aggregated when :

- Unimodal size of the sample is superior to the expected value (+ or – 10 %)
- Polydispersity index is > to 0.12
- particles whose sizes are distinct and superior to the reference size in the same buffer are detected on the SDP intensity bar chart.

## 5. VALIDITY

The assay is valid when the following criteria are met:

### For Size Controls

1. Scattering intensity is between 5.0 E4 and 1.0 E6 counts/sec
2. Dust < 5%
3. Size Control L100 is between 63.4 nm and 77.4 nm with a P.I. < 0.12
4. Size Control L500 is between 386 nm and 522.2 nm with a P.I. < 0.12

### For Z101 reference

1. Scattering intensity is between 5.0 E4 and 1.0 E6 counts/sec
2. Dust < 5%
3. P.I. < 0.12

### For samples

1. Scattering intensity is between 5.0 E4 and 1.0 E6 counts/sec
2. Dust < 5%
3. RSD for unimodal size is < 5%

	<b>Standard operating procedure for the determination of adenovirus aggregation by photon correlation spectroscopy</b>	Reference: N/A
--	--	-------------------