

# ADENOVIRUS TYPE 5 REFERENCE MATERIAL [1]

Produced by the Adenovirus Reference Material Working Group (ARMWG)  
Distributed by ATCC® (Catalog No. VR-1516)

## Description and Background

The Adenovirus Reference Material (ARM) consists of purified Adenovirus, Type 5 (wild type adenovirus, see ATCC VR-5) formulated as a sterile liquid in 20 mM TRIS, 25 mM NaCl, 2.5% glycerol, pH 8.0 at room temperature, and stored frozen at  $-70^{\circ}\text{C}$ . The configuration is 0.5-mL in a Type II glass vial with a Teflon-coated gray butyl stopper, plus an aluminum seal and crimp closure. The U.S. Food and Drug Administration (FDA) has made recommendations for the use of the ARM when characterizing adenoviral gene therapy products [2].

The ARM was developed under the guidance of the Adenovirus Reference Material Working Group (ARMWG) and the U.S. FDA through the donation of services and supplies by a large number of laboratories and organizations from the United States, Canada, France, the Netherlands, Germany, and the United Kingdom [1, 3]. All information regarding the development and characterization of the ARM can be found on The Williamsburg BioProcessing Foundation's website <http://www.wilbio.com>. The purpose of the ARM is to define the particle unit and infectious unit for adenovirus-based gene vectors and establish a reference point for comparisons. The NIH Recombinant DNA Advisory Committee recommended the development of such a reference testing agent in their report issued January 2002 [4].

## Assigned Concentration and Titer

The ARMWG assigned the particle concentration and infectious titer based on statistical analyses [5, 6] of the data derived during the characterization phase of the project. Procedures for obtaining and analyzing these data were provided by the ARMWG (<http://www.wilbio.com>). The particle concentration is  $5.8 \times 10^{11}$  particles/mL, with 95% certainty that the true particle concentration lies within the range of  $5.6 \times 10^{11}$  to  $6.0 \times 10^{11}$  particles/mL. The infectious titer on HEK 293 cells is  $7 \times 10^{10}$  NAS Infectious Units (NIU)/mL, with 95% certainty that the infectious titer on HEK 293 cells lies within the range of  $7 \times 10^{10}$  to  $8 \times 10^{10}$  NIU/mL. The NAS infectious unit is explained in reference [7].

## Recommended Storage and Stability Information

Short-term field use and shipping configuration studies were performed [14]. The field use study focused on the impact of multiple freeze-thaw cycles, thaw and subsequent storage at  $2-8^{\circ}\text{C}$  over 7 days, and thaw and subsequent storage at room temperature over 7 days. Stability studies on the ARM have shown aggregation in some vials after a single freeze-thaw, *i.e.*, after standing for more than 4 hours upon thaw after receipt from ATCC. The ARM can be thawed at room temperature and left at either room temperature or at  $2-8^{\circ}\text{C}$  for as long as 4 hours without impact.

The shipping stability study examined the impact on the ARM when packaged on dry ice using the ATCC shipping configuration, with the package held for two days at  $40^{\circ}\text{C}$  and then an additional day at  $50^{\circ}\text{C}$ . Data confirmed that the ARM could be successfully shipped in the ATCC long distance configuration.

A five-year stability study was initiated in 2001 to monitor the ARM when stored frozen at  $-80^{\circ}\text{C}$ , and, on a more limited basis, when frozen at  $-80^{\circ}\text{C}$  but stored long-term at  $-20^{\circ}\text{C}$ . Stability is being evaluated via: particle concentration by A 260nm in SDS, particle quality and concentration as determined by an anion exchange HPLC assay [15], activity by an infectious titer assay (incorporating the NAS calculation), particle size by photon correlation spectroscopy, particle aggregation by ratio of A 320nm to A 260nm (without SDS), and, for selected time points, a qualitative assessment of micro-aggregation via field emission scanning electron microscopy and assessment of sterility as a correlative for container integrity. Data can be found in Table 1 and Figure 1. Data through the 36 month time point demonstrate that the ARM is stable stored frozen at  $-80^{\circ}\text{C}$ . Long-term storage at  $-20^{\circ}\text{C}$ , however, is not recommended (see Table 2) as both

particle concentration via Resource Q HPLC and infectious titer drop significantly by 12 months; other parameters also indicate a significant change has taken place.

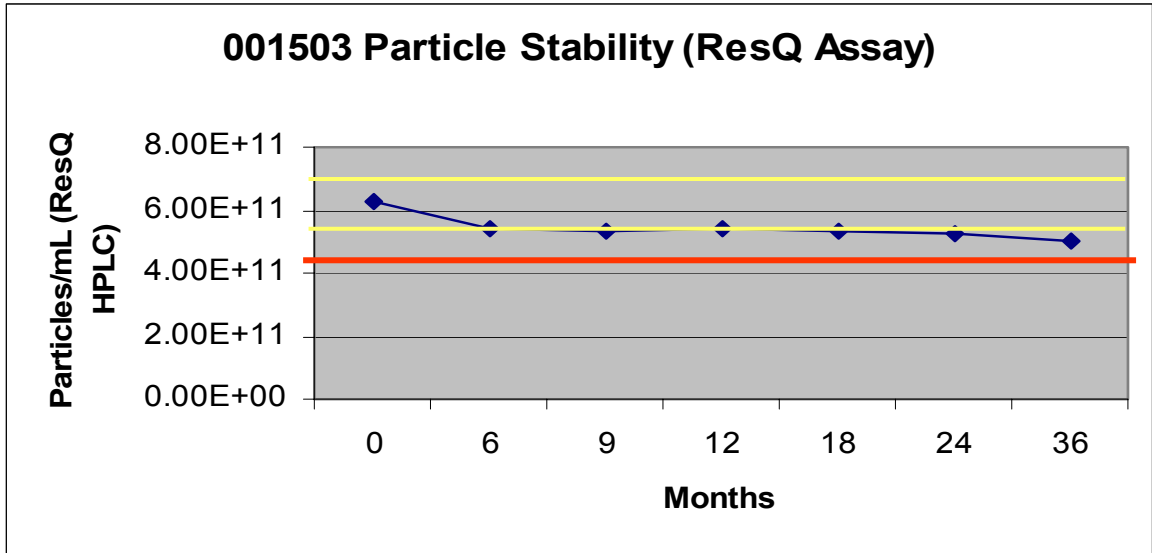
**Table 1 Key -80°C Stability Data for Lot Nos. 001503, 001504, 001505, and 001506**

<b>Assay</b>	<b>Time Point (Month)</b>	<b>Lot No. 001503</b>	<b>Lot No. 001504</b>	<b>Lot No. 001505</b>	<b>Lot No. 001506</b>
Particles/mL via ResQ HPLC [15]	<b>0</b> months (Aug 2001)	6.29 x 10 <sup>11</sup>	6.48 x 10 <sup>11</sup>	6.30 x 10 <sup>11</sup>	6.71 x 10 <sup>11</sup>
	<b>6</b> months (Feb 2002)	5.41 x 10 <sup>11</sup>	Not performed	Not performed	Not performed
	<b>9</b> months (May 2002)	5.32 x 10 <sup>11</sup>	Not performed	Not performed	Not performed
	<b>12</b> months (Aug 2002)	5.44 x 10 <sup>11</sup>	5.50 x 10 <sup>11</sup>	5.52 x 10 <sup>11</sup>	5.57 x 10 <sup>11</sup>
	<b>18</b> months (Feb 2003)	5.34 x 10 <sup>11</sup>	Not performed	Not performed	Not performed
	<b>24</b> months (Aug 2003)	5.24 x 10 <sup>11</sup>	Not performed	Not performed	Not performed
	<b>36</b> months (Jul 2004)	5.00 x 10 <sup>11</sup>	5.04 x 10 <sup>11</sup>	5.18 x 10 <sup>11</sup>	5.17 x 10 <sup>11</sup>
	<b>48</b> months (Jul 2005)	Pending	Pending	Pending	Pending
	<b>60</b> months (Aug 2006)	Pending	Pending	Pending	Pending
Infectious Titer (NAS IU/mL)	<b>0</b> months (Aug 2001)	3.4 x 10 <sup>11</sup>	2.5 x 10 <sup>11</sup>	2.5 x 10 <sup>11</sup>	2.2 x 10 <sup>11</sup>
	<b>6</b> months (Feb 2002)	2.4 x 10 <sup>11</sup>	Not performed	Not performed	Not performed
	<b>9</b> months (May 2002)	1.1 x 10 <sup>11</sup>	Not performed	Not performed	Not performed
	<b>12</b> months (Aug 2002)	3.9 x 10 <sup>11</sup>	2.9 x 10 <sup>11</sup>	2.9 x 10 <sup>11</sup>	2.2 x 10 <sup>11</sup>
	<b>18</b> months (Feb 2003)	1.7 x 10 <sup>11</sup>	Not performed	Not performed	Not performed
	<b>24</b> months (Aug 2003)	5.2 x 10 <sup>11</sup>	Not performed	Not performed	Not performed
	<b>36</b> months (Jul 2004)	<sup>a</sup> Not available	Not performed	Not performed	Not performed
	<b>48</b> months (Jul 2005)	Pending	Pending	Pending	Pending
	<b>60</b> months (Aug 2006)	Pending	Pending	Pending	Pending

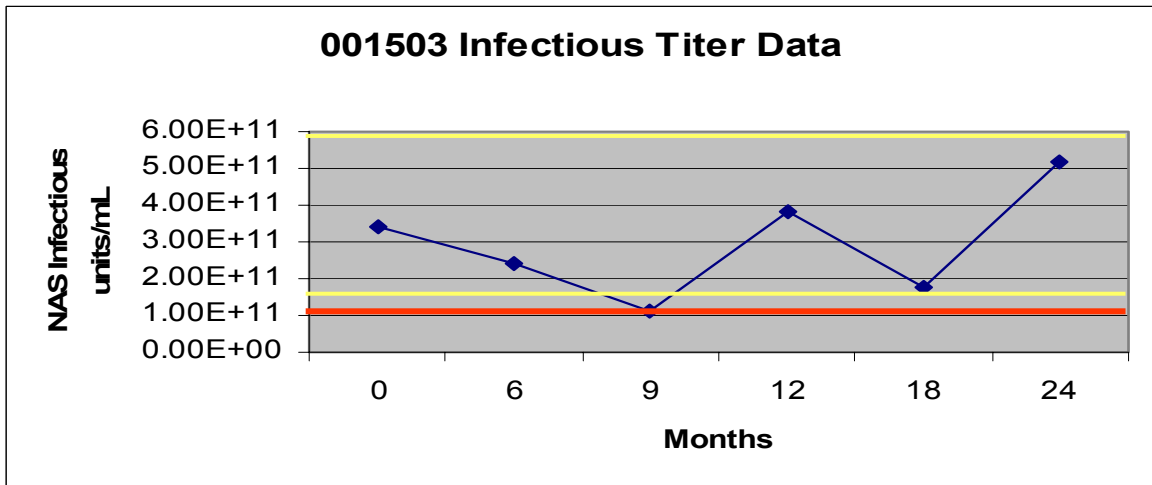
a: Assay not valid.

**Figure 1.** **-80°C Stability of Lot No. 001503.** Yellow lines indicate upper and lower limits for stability. Red line indicates value below which the material may no longer be considered as stable. A: Particle assay data. B: Infectious titer assay data.

A.



B.



**Table 2 Key -20°C Stability Data for Lot No. 001503**

<b>Assay</b>	<b>Time Point (Month)</b>	<b>Lot No. 001503 Data</b>
Particles/mL via ResQ HPLC [15]	<b>0</b> months (Sep 2001)	6.29 x 10 <sup>11</sup>
	<b>12</b> months (Nov 2002)	<3.09 x 10 <sup>10</sup> 3.37 x 10 <sup>11</sup>
	<b>24</b> months (Nov 2003)	<3.09 x 10 <sup>10</sup> <3.09 x 10 <sup>10</sup>
Particles/mL via OD 260nm / SDS ARMWG SOP	<b>0</b> months (Sep 2001)	6.34 x 10 <sup>11</sup>
	<b>12</b> months (Nov 2002)	4.39 x 10 <sup>11</sup>
	<b>24</b> months (Nov 2003)	4.02 x 10 <sup>11</sup>
Infectious Titer (NAS IU/mL)	<b>0</b> months (Sep 2001)	3.4 x 10 <sup>11</sup>
	<b>12</b> months (Nov 2002)	<2.4 x 10 <sup>7</sup> <9.0 x 10 <sup>0</sup>
	<b>24</b> months (Nov 2003)	8.7 x 10 <sup>8</sup> <2.4 x 10 <sup>7</sup>
Particle Size (nm)	<b>0</b> months (Sep 2001)	112.6
	<b>12</b> months (Nov 2002)	120.0
Virus Aggregation via OD 320nm / 260nm	<b>0</b> months (Sep 2001)	0.22
	<b>12</b> months (Nov 2002)	0.27
	<b>24</b> months (Nov 2003)	0.43
Bioburden (Vial integrity surrogate)	<b>0</b> months (Sep 2001)	Negative
	<b>15</b> months (Dec 2002)	Negative

## References:

All information regarding the development and characterization of the Adenovirus Reference Material can be found on The Williamsburg BioProcessing Foundation's website <http://www.wilbio.com>. The ARMWG has published many of the details in the scientific literature.

1. Beth Hutchins (January 2002) "Development of a Reference Material for Characterizing Adenovirus Vectors," *BioProcessing Journal* 1 (1): 25-28
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4. NIH Recombinant DNA Advisory Committee (January 2002) "NIH Report: Assessment of Adenoviral Vector Safety and Toxicity: Report of the National Institutes of Health Recombinant DNA Advisory Committee," *Human Gene Therapy* 13 (1): 3-13
5. Janice D. Callahan (May 2002) "Statistical Analysis of Adenovirus Reference Material Assay Results: Determination of Particle Concentration and Infectious Titer," Callahan Associates Inc. (La Jolla, CA)
6. Janice D. Callahan (November 2002) "A Statistical Analysis of Adenovirus Reference Material Assay Results," *BioProcessing Journal* 1 (3): 43-47
7. Cassandra Nyberg-Hoffman, Paul Shabram, W. Lei, Daniel Giroux, and Estuardo Aguilar-Cordova (1997) "Sensitivity and reproducibility in adenoviral infectious titer determination," *Nature Medicine* 3: 808-811
8. Kodjo Adadevoh, Maria Croyle, Daniel Malarme, Edwige Bonfils, and Mark A. Bowe (November 2002) "A Short-Term Field Use and Shipping Stability Study of a Wild Type Ad5 Adenoviral Reference Material," *BioProcessing Journal* 1 (3): 62-69
9. Paul W. Shabram, Daniel D. Giroux, Ann M. Goudreau, Richard J. Gregory, Mark T. Horn, Bernard G. Huyghe, Xioadong Liu, Mary H. Nunnally, Barry J. Sugarman, and Suganto Sutjipto (1997) "Analytical anion-exchange HPLC of recombinant type-5 adenovirus particles," *Human Gene Therapy* 8: 453-465

## Key Abbreviations Used

ARMWG – Adenovirus Reference Material Working Group  
ATCC – American Type Culture Collection  
FDA-CBER – Food and Drug Administration - Center for Biologics Evaluation and Research  
IND – Investigational New Drug (application to U.S. FDA)  
NAS – Normalized and Adjusted Standard; see reference 7  
NIH – National Institutes of Health, U.S.  
NIU/mL – NAS corrected infectious units per milliliter  
RCA – replication competent adenovirus  
ResQ HPLC – Resource Q anion exchange high pressure liquid chromatography assay