Adenoviral Reference Material Working Group
Bid Submission Form – Purified Formulated Bulk Virus Reference Material Production and Release Testing Donation
RFP 5.0

Item for Submission

Production and release testing of an Ad5 Wild-type Virus Bulk Reference Material in final formulation ready to be vialed. The bidder will also provide a Certificate of Analysis summarizing characterization as called for below. The intended lot size for the reference material is to enough bulk for vialing of 4500 to 5000 x 0.5 mL vials at 2 to 5 x 10^{11} particles/mL, or equivalent to approximately 1.3 x 10^{15} total particles after release. The bulk material should be delivered in at least 4 aliquots.

General Requirements for Bidding

Reference material will need to be produced under conditions equivalent to CGMP. Virus bank and cell bank vials used for reference material production will be supplied from other bid activities in this process. Indicate your minimum requirements / concerns for acceptance of these materials (cell bank vials and viral bank vials) if not addressed by characterization called for RFP 4.0. The bid should indicate the amount of time required from receipt of the cell bank vials and viral bank vials for the production and release of the purified, formulated bulk reference material.

The institution will need to provide a brief statement describing the proposed method for production and purification of the reference material bulk, including proposed container configuration. The institution should include their proposed specifications, including information on the proposed test methods, and proposed Certificate of Analysis addressing the following points:

?? Identity
?? Purity
?? Provisional Particle concentration
?? Functional activity (such as infectivity)
?? Sterility (USP or 21CFR610.12)
?? Mycoplasma
?? Endotoxin
?? *In vitro* adventitious viral agents or equivalent
?? AAV

And where applicable

?? Bovine virus – Certificate of Analysis (raw material or final vial test) (per 9CFR113.47)
?? Porcine parvovirus – Certificate of Analysis (raw material trypsin or final vial test)

The proposal should include details of the proposed method/container for shipping to ensure integrity of the bulk material upon arrival at the vialing facility. The proposal should also include instructions regarding how the material is to be dispensed. Examples are "the material will be shipped at 2-8?C and should be dispensed before freezing," or, "the material will be shipped frozen; it should be thawed under the following conditions (supplied) and then should be dispensed and then frozen."

In addition to these specific documentation requirements, each institution should include a brief statement describing their experience and capacity to perform this activity and a description of the facility in which the work will be performed. The facility description should address procedures to ensure segregation during viral bulk reference material production and purification.

It is expected that the final documentation package made available with the formulated bulk would include copies of the completed batch records used for production.

Documentation Requirements

Documentation should include detailed information on a proposed final formulation, which should not be solely PBS (phosphate buffered saline)-based nor should it contain protein. This information should include supporting data indicating the formulation's ability to provide stability for storage of Adenovirus at $\leq$-55?C. The formulation information should also indicate compatibility with biological and chemical characterization methods. The working group will determine the final formulation used.

The bid should include a description of the proposed cell and viral culture, harvest, and purification methods along with the proposed specifications, test methods, and proposed Certificate of Analysis.

The proposal should include details of the proposed method/container for shipping the formulated bulk reference material to the vialing facility.

Please complete the following fields:

Contact Information – RFP 5.0

Contact Individual:	Joe Senesac
Institution:	Introgen Therapeutics, Inc.
Address:	2250 Holcombe Blvd. Houston, Texas 77030
Phone Number:	(713) 610-4020
Email Address:	j.senesac@introgen.com

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Indicate Propagation Method: ☐ Suspension ☒ Adherent

Please indicate if your institution is also submitting proposals for the other activities:

- ☐ Donation of Cell Bank
- ☐ Donation of Ad5 Wild-type Virus
- ☒ Ad5 Wild-type Virus Bank Production
- ☐ Donation of Repository Services
- ☒ Vialing of Ad5 Wild-type Reference Material
- ☐ Donation of Supplies/Other Services

Please attach:
Proposed Certificate of Analysis, specifications, and test methods
Proposed method for production and purification
Proposed Formulation Information
Institution Capability Statement
Information on shipping

Submit this completed form and all attached information for receipt **by February 28, 2001** to the address below. Electronic submissions are encouraged. Final decisions will be communicated by or about March 31, 2001. Please note that all information submitted will be publicly available. Please do not mark any information confidential, as we cannot honor that request. Please also include an estimate of cost and market value of donated goods and services.

Williamsburg BioProcessing Foundation
Attn: Adenovirus Reference Material Working Group
4015 Killam Avenue
Norfolk, VA 23508

PH: 757-423-8823
FAX: 757-423-2065

EMAIL: advector@wilbio.com

A. Capability Statement

Introgen Therapeutics, Inc. (Company) is engaged in the manufacture, research and clinical development of viral vector-based gene therapies for cancer treatment. The Company utilizes a 12,000 square foot manufacturing facility at 2252 Holcombe Boulevard in Houston, TX for the production of viral vectors for non-clinical and clinical studies. This facility is fully commissioned and has been qualified via the production of three lots of an adenoviral vector, RPR/INGN 201, containing a functional copy of the human *p53* gene Ad5CMV-*p53*.

Introgen has produced over 30 clinical batches of various materials for Phase I to Phase III clinical studies. The facilities include class 100,000 down to Class 1,000 cleanrooms which provide two separate manufacturing suites and appropriate environments for Cell/Viral Culture, Purification, and Finishing activities. Key members of the manufacturing team have worked together at Introgen for greater than 5 years. Introgen also has fully staffed Quality Assurance and Quality Control departments for testing and oversight of production.

B. Production Requirements

Cell Bank Vials

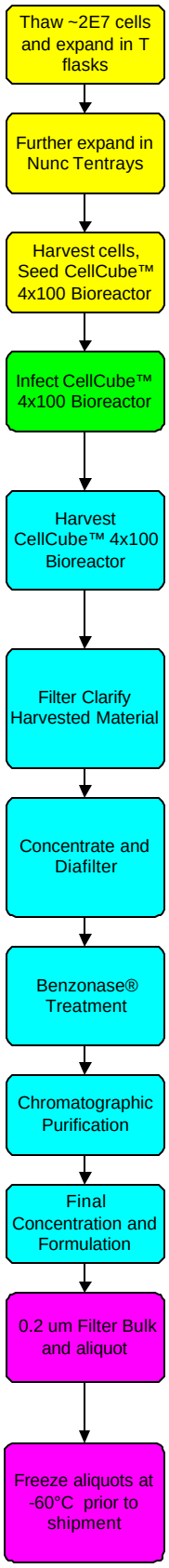
Production will require a minimum of 4E7 total viable adherent cells in multiple vials. Vials should be shipped to Introgen in the vapor phase of Liquid Nitrogen. Required documentation will include the completed Certificate of Analysis as specified in RFP 1.0, and a description of cell culture techniques specific to the cell line. Cell culture media is to be defined as to vendor and catalog number.

Virus Bank Vials

Production will require a minimum of 4E12 viral particles to be shipped to Introgen on dry ice. Required documentation will include the completed Certificate of Analysis as specified in RFP 4.0.

C. Formulated Bulk Virus Production

Production Flowchart


Thaw ~2E7 cells and expand in T flasks
Further expand in Nunc Tentrays
Harvest cells, Seed CellCube™ 4x100 Bioreactor
Infect CellCube™ 4x100 Bioreactor
Harvest CellCube™ 4x100 Bioreactor
Filter Clarify Harvested Material
Concentrate and Diafilter
Benzonase® Treatment
Chromatographic Purification
Final Concentration and Formulation
0.2 um Filter Bulk and aliquot
Freeze aliquots at -60°C prior to shipment

General information

Cell culture operations take place in a class 10,000 cleanroom equipped with a class 100 biosafety cabinet (BSC). Bioreactor culture and downstream processing will take place in a class 100,000 cleanroom equipped with class 100 BSCs and portable ULPA laminar air stations. All equipment and HEPA filters have current calibration or certification. The production in the facility is campaigned, with no overlap between lots of material produced in a suite, and with the rooms thoroughly cleaned and disinfected at the end of production and prior to beginning the next batch. Production personnel are limited to working with a single construct per working day. Production materials are sterile single-use disposables wherever possible. Equipment that is not disposable is thoroughly decontaminated and cleaned prior to being sterilized or sanitized for use.

Cell/Viral Culture

Cells are thawed at 37?C prior to placement in a BSC and transfer to sterile disposable culture flasks. The adherent cells will be expanded in a timeframe based on the directions of the cell bank donor. Expansion will start in T flasks and will progress through the use of disposable Nunc Cell Factories until a cell mass is reached that is sufficient to seed the CellCube™ 4x100 Bioreactor. It is anticipated that a single CellCube™ 4x100 Bioreactor run will produce sufficient product for the scope of this project. The CellCube™ 4x100 Bioreactor and tubing set are disposable. The associated oxygenator and probes are reusable.

The cells will be allowed to expand for a predetermined number of days prior to infection. To infect the CellCube™ 4x100 Bioreactor, the virus bank is injected via an access port. The bioreactor is incubated for a predetermined number of days prior to harvest.

Harvest and Downstream Processing

The harvested material will be filter clarified prior to concentration and diafiltration. Benzonase® treatment to reduce residual host cell nucleic acids will also be performed prior to purification.

Column chromatography will be used to purify the material, which will then be further concentrated and formulated into the designated formulation. The final formulation will be prepared with specified USP chemicals and USP water for injection.

The formulated bulk will be 0.2 um filtered and aliquoted prior to freezing at ? -60?C.

D. Production Schedule

After receipt of the cell bank vials a three-week trial period would be necessary to assess cell growth characteristics and to develop documentation. The production

of the formulated Bulk product would require approximately one month. Release testing will require a period of 12 weeks.

E. Final Formulation and Container/Shipment

The final formulation is 20mM TRIS, pH 8.0, 2.5% Glycerol, 25mM NaCl, which has been donated by Selective Genetics. This formulation has demonstrated good stability characteristics as detailed in the attached Formulation file.

The Bulk material will be sterile filtered and aliquoted into sterile 50mL polypropylene tubes for shipment. An estimated 50-60 tubes will be filled and appropriately labeled prior to freezing at ? -60?C.

Shipment of the aliquots will be on dry ice with the addition of a calibrated temperature trace device to document shipping conditions. The shipping container will be an EnduroTherm® insulated container.

The material should be thawed at room temperature prior to sterile filtration and product fill.

F. Certificate of Analysis and Specifications

See attached proposed Certificate of Analysis containing specifications.

G. Documentation

A detailed production report will be submitted upon completion of testing. The report will document the production methods and will include the completed Certificate of Analysis. This will be made available in lieu of completed production batch records.

Introgen Therapeutics, Inc.
Certificate of Analysis for Formulated Bulk Adenovirus Serotype 5
Reference Material
Lot #

Manufactured By: Introgen Therapeutics, Inc.
Adenovirus Serotype 5 Reference Material
Date of Manufacture:
Virus Bank:

Lot Number:
Volume
Store at -60°C or below
Cell Bank:

Test	Sponsor	Specification	Result
Pre-Infected Cells			
In-vitro Adventitious Virus	TBD*	No evidence of adventitious viral agents	
Crude Cell Lysate			
Mycoplasma EP & PTC 1993	MDS Panlabs	Negative for presence of Mycoplasma	
Bioburden	TBD*	Report Value	
Adeno-Associated Virus (PCR)	TBD*	Negative for Adeno-associated Virus	
Purified Bulk Product (Postfilter)			
Sterility USP & EP	MDS Panlabs	Sterile	
Bacterial Endotoxins Test	Introgen	Report Value	
Plaque Titration of Adenovirus Vector	Introgen	Report Value	
Virus Particle Enumeration by OD_{260}	Introgen	2E11 – 5E11 vp/mL	
Purity by HPLC Ion Exchange	Introgen	? 98% Purity	
Bovine Serum Albumin (ELISA)	Introgen	Report Value	
huDNA	TBD*	Report Value	
pH	Introgen	7.6 – 8.4	

I certify that the above information has been accurately transcribed.

By: _____ _____
Quality Assurance Representative Date

* Testing will be performed at a CGMP facility but Introgen has not yet determined which test site will be used.

As part of RFP 5.0, Mark D'Andrea (Senior Director, Process Development), representing Selective Genetics, Inc. (11035 Roselle St., San Diego, CA, 92121), respectfully submits an aqueous, Tris-buffered formulation containing glycerol and NaCl for use as the final formulation of the purified reference standard material. The following details the composition of the formulation and provides stability data demonstrating the usefulness of this specific formulation.

1. Summary

The stability of first generation (E1, E3 deleted) recombinant adenovirus type 5 encoding the PDGF-B (Platelet-derived growth factor B) gene, was studied by subjecting formulated, purified virus to freeze-thaw and temperature stress at various adenovirus concentrations from $1.7x10^{10}$–$1.7x10^{12}$ PN/ml (adenoviral particle number per milliliter). The GTS formulation (2.5% glycerol v/v, 20 mM Tris pH 8, 25 mM NaCl) provided good stability for adenovirus at all particle concentrations tested from $1.7x10^{10}$ to $1.7x10^{12}$/ml, whether stored in plastic or glass vials.

At temperatures of 2-8?C and higher, virus stability was dependent on particle concentration. At the highest concentration ($1.7x10^{12}$ PN/ml), a 15% and 65% decrease in particle recovery was observed at 3 and 6 months, respectively. At concentrations equal to or lower than $1.7x10^{11}$ PN/ml, GTS formulated adenovirus has remained stable for at least 6 months when stored at 2-8°C in cryovials. Concentrations = $5.1x10^{11}$ PN/ml contained in glass vials have shown stability at 2-8°C for at least 3 months.

2. Stability indicating methods

Analytical methods including anion exchange HPLC (AE-HPLC), Laser light scattering (LLS) and a bioactivity (ELISA) assay were developed as stability indicating methods. AE-HPLC and functional gene expression assays were used to examine the structural integrity and activity of adenovirus. Laser light scattering (LLS) was used to monitor viral particle aggregation, an early event in purified virus degradation.

AE-HPLC Particle Determination
Particle concentrations were analyzed by AE-HPLC utilizing a Resource Q column attached to an HP1050 HPLC. Using this method, intact adenoviral particles are quantified at a detection limit of ca. $2x10^{10}$ PN/ml, and a limit of quantitation of ca. $3.8x10^{10}$ PN/ml. Particle concentrations are calculated by comparing average peak areas to a calibration curve prepared from the analysis of a reference standard.

Laser Light Scattering (LLS)
The average virus particle size is determined by LLS using a Zetasizer 5000 (Malvern Instruments, England). The average hydrodynamic diameter (Z_{ave}, nm) is measured with an argon-ion laser operating at 488 nm, 15 to 50 milliwatts, and a 90? angle. The hydrodynamic diameter (Z_{ave}) describes the apparent size of the particle as it exists in the solution, and is reported as the average size of the entire population. Adenoviral vectors have an apparent size range of 80-120 nm. The homogeneity (or polydispersity) of the population is reported using a polydispersity index which indicates the particle size

distribution within the population. As the polydispersity index approaches 1.0, the sample is more likely to contain multiple populations of varying Z_{ave}. A monodisperse distribution will have a polydispersity index of <0.2.

AdPDGF-B Transduction/PDGF-BB Expression
To confirm PDGF-BB expression, 293 cells are transduced and cell lysate supernatant subjected to ELISA analysis. ELISA plates (96-well) are coated with the human PDGF receptor ?/Fc chimera which captures the PDGF-BB dimer. Recombinant human PDGF-BB protein is used as a positive control. Activity is quantitated using the concentration of AdPDGF-B which produces 50% maximal response (effective concentration-50; EC_{50}) in PN/cell, or nanograms PDGF-BB at a specific PN/cell ratio.

3. Experimental
Lot A:
Aliquots of Lot A were filled into sterile 1.8 ml cryovials at 3 different viral particle concentrations: $1.7x10^{12}$ PN/ml, $1.7x10^{11}$ PN/ml and $1.7x10^{10}$ PN/ml. For freeze-thaw testing, high and mid-concentration samples were frozen in an ethanol-dry ice bath for 5 minutes and thawed at room temperature (20-25?C) for 15-20 min. For long-term temperature stability studies, vials were stored in temperature controlled environments.

Lot B:
Aliquots of Lot B were filled into sterile 3 ml, 13 mm serum/lyophilization vials, sealed with 13 mm grey butyl stoppers and crimped with flip-off button crimps. Lot B was formulated at high, middle and low particle concentrations: $5.1x10^{11}$ PN/ml, $1.7x10^{11}$ PN/ml and $5.1x10^{10}$ PN/ml, respectively. For freeze-thaw studies, each vial was frozen in a -20?C non-cycling freezer for ?3 hours and thawed at 20-25?C for 30-60 min. For long-term temperature stability studies, vials were stored in temperature controlled environments.

4. Results:
Freeze-Thaw Stability
As shown in Tables 1 and 2, the particle size and polydispersity of samples for both Lots A and B remained unchanged following 1, 3, and 5 freeze-thaw cycles. The low particle concentration samples of Lot A and Lot B precluded LLS analysis. Similarly, AE-HPLC results indicated quantitative recovery of intact particles (Figures 1A and 2A). Lastly, no changes in PDGF-BB expression, as compared to a fresh control (0 F-T), were demonstrated by AdPDGF-B transduction/PDGF-BB ELISA analysis (Figure 1B, and 2B). These data indicate that both AdPDGF-B Lots remain stable for at least 5 freeze-thaw cycles whether stored in plastic or glass vials.

Table 1. Particle Size and Polydispersity of Lot A Subjected to Freeze-Thaw Stress

# Freeze-thaws cycles	**0**		**1**		**3**		**5**	
	Z_{ave}	Poly-dispersity	Z_{ave}	Poly-dispersity	Z_{ave}	Poly-dispersity	Z_{ave}	Poly-dispersity
$1.7x10^{12}$ PN/mL	106	0.06	102	0.12	101	0.12	101	0.11
$1.7x10^{11}$ PN/mL	100	0.10	102	0.10	100	0.08	101	0.10

Figure 1. AE-HPLC and PDGF-BB Expression Analysis of Lot A Subjected to Freeze-Thaw Stress

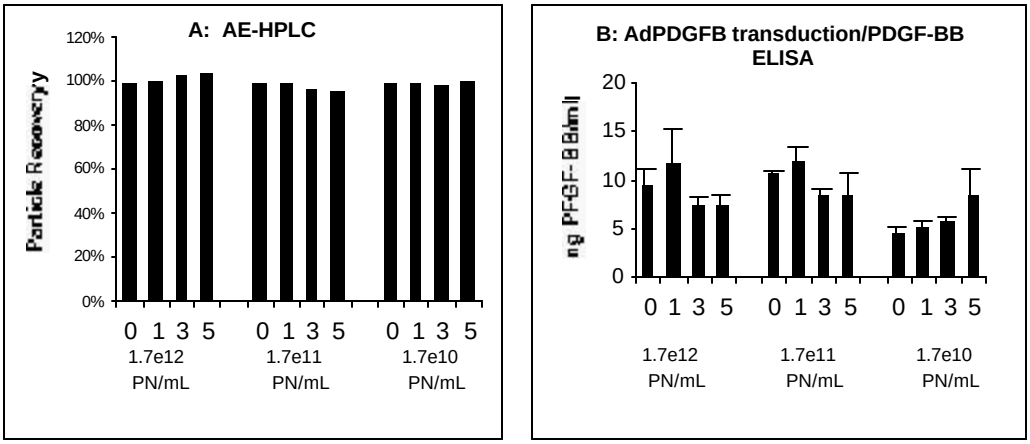
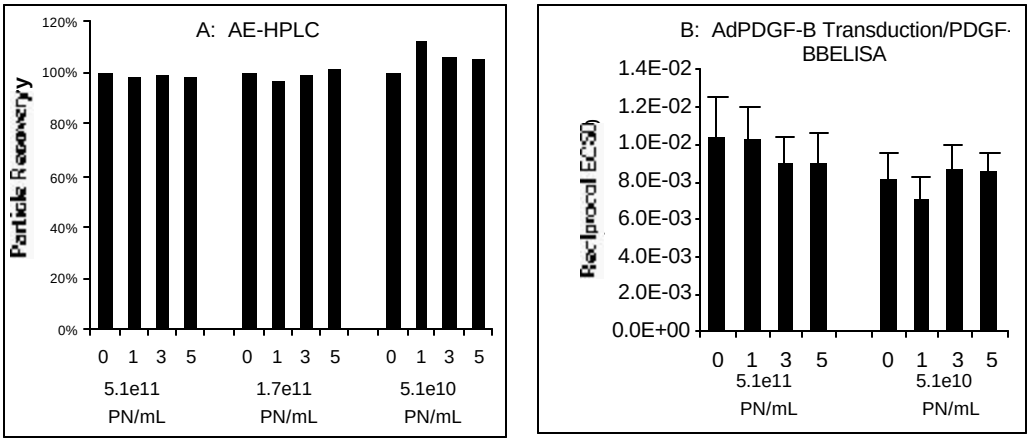


Table 2. Particle Size and Polydispersity of Lot B Subjected to Freeze-Thaw Stress

# Freeze thaw cycles:	0		1		3		5	
	Z_{ave}	Poly-dispersity	Z_{ave}	Poly-dispersity	Z_{ave}	Poly-dispersity	Z_{ave}	Poly-dispersity
$5.1x10^{11}$ PN/mL	104	0.06	103	0.06	104	0.04	103	0.05
$1.7x10^{11}$ PN/mL	105	0.05	105	0.10	105	0.08	103	0.11

Figure 2. AE-HPLC and PDGF-BB Expression Analysis of Lot B Subjected to Freeze-Thaw Stress



Temperature Stability

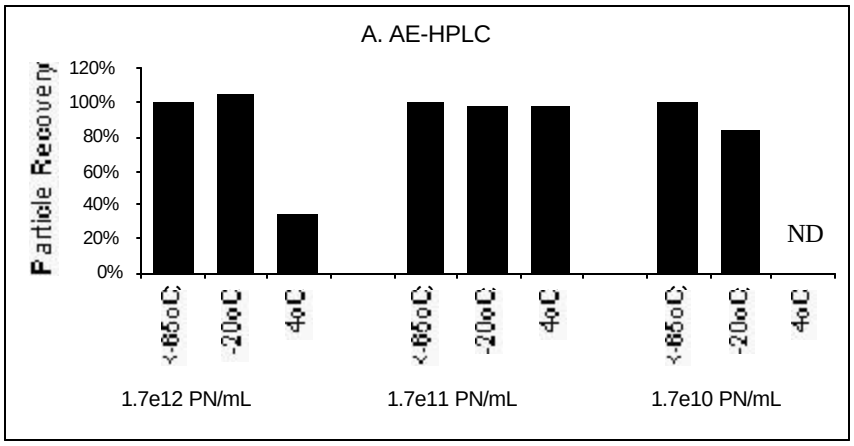
A six month temperature stability profile for Lot A was determined. Similar temperature stability studies for Lot B up to 3 months storage have been completed. Additional timepoints will be analyzed as the Lot B test articles become available. Test samples were stored at ≤ -65°C (control, data to which other test articles are compared), -20?C,

and 2-8°C. In addition, for Lot A, accelerated stability studies were performed at 35°C at the high and middle particle concentrations.

Temperature Stability of Lot A

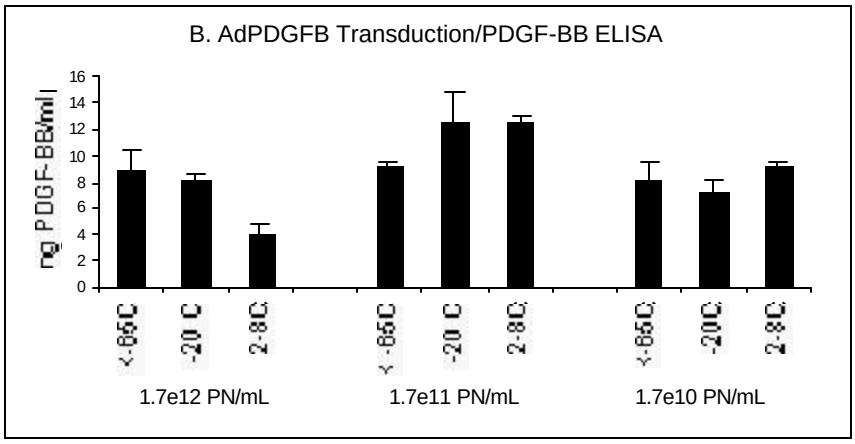
Lot A samples were analyzed at 2 weeks, 1 month, 3 month, and 6 month time points. The vector remained stable for at least 3 months, as determined by LLS, AE-HPLC analysis, and AdPDGF-B transduction/PDGF-BB ELISA. The 6-month stability data indicate all samples were stable at -20?C. Middle and low concentration samples were stable at 2-8?C. However, the high dose 2-8?C sample showed a >50% decrease in intact particle recovery and PDGF-BB expressed, as determined by AE-HPLC and AdPDGF-B transduction/PDGF-BB ELISA analyses (Figure 3 and Figure 4, respectively). Particle size remained at about 100 nm suggesting that no aggregation had occurred. The overall intensity of scattered light decreased ca. 40% in these samples, indicating loss of viral particles. The decrease of intact AdPDGF–B particles and PDGF-BB expression appears to be due to viral particle disintegration.

Figure 3. Total Particle Recovery (AE-HPLC) of Lot A Stored for 6 Months



ND- Not Determined

Figure 4. AdPDGF-B Transduction/PDGF-BB ELISA Analysis of Lot A Stored for 6 Months



A 2-week accelerated stability study was performed at 35°C. AE-HPLC and PDGF-BB ELISA were performed to determine the vector integrity and bioactivity, respectively. The middle-particle concentration, $1.7x10^{11}$ PN/ml, remained stable for 7 days, and showed a loss of particle recovery and a decrease in transgene expression at 14 days. At the higher particle concentration, $1.7x10^{12}$ PN/ml, there is a time dependent decrease in particle recovery and transgene expression beginning at day 1.

Temperature Stability of Lot B

Long-term temperature stability studies for Lot B are currently in progress. Analysis of LLS data demonstrated no changes in particle size or average size distribution for 3-month incubation at all temperatures. AE-HPLC indicated quantitative recovery of particles for all three concentrations for all three temperature conditions. The biological activity of the high concentration sample ($5.1x10^{11}$ PN/mL) was stable at 3 months. The biological activity of the middle concentration sample ($1.7x10^{11}$ PN/mL) could not be determined due to an artifactual low response of the positive reference (-65°C) control. Future stability determinations are planned for these samples in an ongoing study.