
Product Manual

AAV-2 Helper Free Bicistronic Expression System (GFP)

Catalog Number

VPK-418-SER2

1 kit

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Adeno-associated viruses (AAVs) are derived from defective parvoviruses, which depend on essential helper functions provided by other viruses, such as adenovirus and herpes virus, for efficient viral replication and propagation. AAV has no etiologic association with any known diseases and has been successfully used to establish efficient and long-term gene expression *in vivo* in a variety of tissues without significant cellular immune responses or toxicity.

AAV has a single-stranded DNA genome which consists of approximately 4.7 kb. All characterized AAV serotypes share three key features, including two copies of AAV terminal repeats (ITRs), one *rep* region and one *cap* region. The ITRs are capable of forming T-shape secondary structure and are the only *cis* elements that are required for AAV replication, packaging, integration, and rescue. The *rep* region encodes four overlapping proteins designated as Rep78, Rep68, Rep52, and Rep40, according to the apparent molecular mass of the protein. In addition to their well-defined roles in AAV replication, Rep proteins also regulate AAV packaging and site-specific integration. The *cap* region encodes three structural proteins, VP1, VP2, and VP3. These three proteins share the same reading frame (see Figure 1).



Figure 1. Schematic Map of AAV Genome. Rep: involved in genome replication; VP1/2/3: capsid proteins.

Cell Biolabs' AAV Helper-Free System allows the production of infectious recombinant human adeno-associated virus (rAAV) virions without the use of a helper virus (Figure 2). In the AAV Helper-Free System, most of the adenovirus gene products required for the production of infective AAV particles are supplied on the plasmid pHelper (i.e. E2A, E4, and VA RNA genes) that is co-transfected into cells with human AAV vector DNA. The remaining adenoviral gene product is supplied by the 293 host cells, which stably express the adenovirus E1 gene. By eliminating the requirement for live helper virus the AAV Helper-Free System provides a safer and more convenient gene delivery system. In the AAV Helper-Free System, the *rep* and *cap* genes have been removed from the viral vector that contains AAV-2 ITRs and are supplied *in trans* on the plasmid pAAV-RC. The removal of the AAV *rep* and *cap* genes allows for insertion of a gene of interest in the viral genome. Cell Biolabs' The AAV Helper-Free System can accommodate inserts of up to 3 kb (See Table 1 for detail).

Recombinant adeno-associated viruses are important tools for gene delivery and expression. AAV has not been reported to cause any diseases. Together with its replication defective nature, AAV has good

safety profile to be used in gene transfer in vivo, and as potential gene therapy vehicles. Recombinant AAV is capable of infecting a broad range of cell types including non-dividing cells and remaining as concatemers for long-term expression. Compared with other viral vectors such as adenovirus, AAV elicits very mild immune response in animal models.



Figure 2. AAV Helper-Free System.

Catalog #	Product Name	Capacity (kb)
VPK-410	pAAV-MCS	3
VPK-415	pAAV-IRES-Puro	1.8
VPK-416	pAAV-IRES-Neo	1.6
VPK-417	pAAV-IRES-Hygro	1.4
VPK-418	pAAV-IRES-GFP	1.7
VPK-419	pAAV-IRES-Bsd	2

Table 1. Packaging capacity of AAV shuttle vectors.

Related Products

1. VPK-402: AAV-2 Helper Free Packaging System
2. VPK-410-SER2: AAV-2 Helper Free Expression System
3. VPK-415-SER2: AAV-2 Helper Free Bicistronic Expression System (Puro)
4. VPK-418: pAAV-IRES-GFP Expression Vector
5. AAV-100: 293AAV Cell Line
6. VPK-140: ViraBind™ AAV Purification Kit
7. VPK-141: ViraBind™ AAV Purification Mega Kit
8. VPK-145: QuickTiter™ AAV Quantitation Kit
9. AAV-200: ViraDuctin™ AAV Transduction Kit

Kit Components

1. pAAV-IRES-GFP Expression Vector (Part No. VPK-418): One 40 µL vial at 0.25 mg/mL.
2. pAAV-RC2 Vector (Part No. VPK-422): One 40 µL vial at 0.25 mg/mL.
3. pHelper Vector (Part No. 340202): One 40 µL vial at 0.25 mg/mL.
4. pAAV-GFP Control Vector (Part No. AAV-400): One 40 µL vial at 0.25 mg/mL.

Materials Not Supplied

1. 293 cells: we recommend 293AAV Cell Line (Cat.# AAV-100) for high titer production of AAV.
2. Cell Culture Medium
3. Transfection Reagents
4. 0.5 M EDTA

Storage

Upon receipt, store all other kit components at -20°C until their expiration dates.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. The AAV Helper-Free system is designed to minimize the chance of generating wild type AAV, but precautions should still be taken to avoid direct contact with viral supernatants.

Vector Features

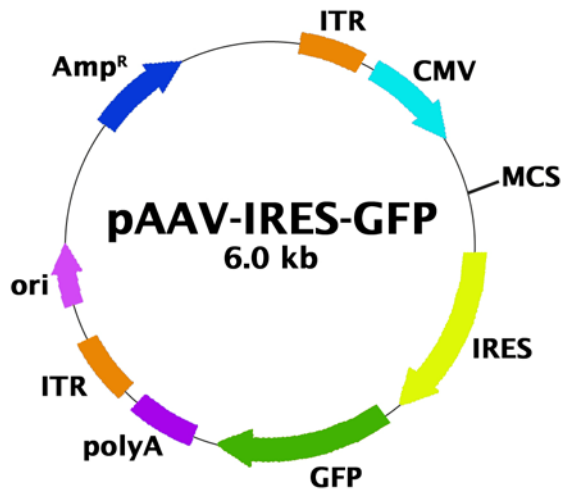


Figure 3: pAAV-IRES-GFP Expression Vector (see Appendix for more detail).

Vector Features:

1 ~ 130:	Left ITR
139 ~ 801:	CMV Promoter
809 ~ 1301:	human β -globin intron
1308 ~ 1376:	MCS
1407 ~ 1985:	IRES
1986 ~ 2705:	GFP
2717 ~ 3195:	PolyA
3235 ~ 3375:	Right ITR
4292 ~ 5152:	Ampicillin Resistance

MCS:

- Enzyme Sites: 5' - ClaI, BamHI, EcoRV, XhoI, EcoRI, XhoI -3'
- MCS Sequence:

CATCGATTGAATTGGATCCGATATCTAGACAGAAGCTTGACCTCGAGCACGAATTCCTGCAGGCCTCGAGG

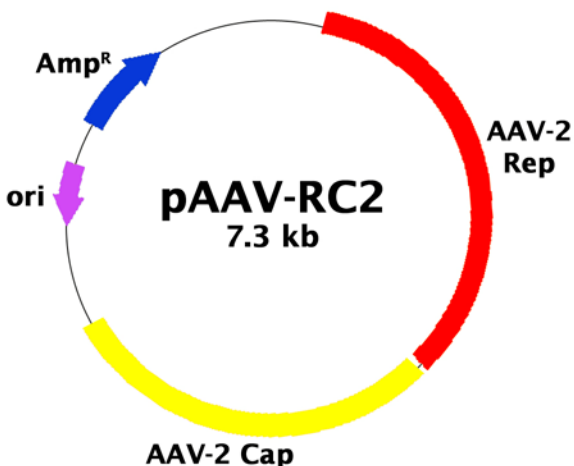


Figure 4. pAAV-RC2 Vector

Vector Features:

131 ~ 1996:	AAV-2 Rep
2013 ~ 4220:	AAV-2 Cap
5292 ~ 6152:	Ampicillin Resistance

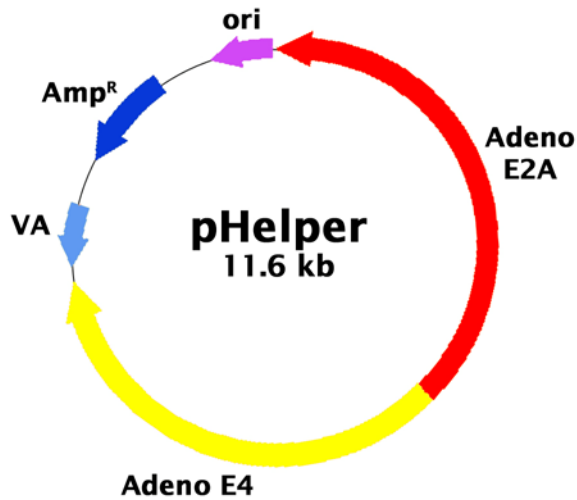


Figure 5. pHHelper Vector

Vector Features:

- 1 ~ 5336: Adeno E2A
- 5337 ~ 8537: Adeno E4
- 8535~ 9280: Adeno VA
- 10182 ~ 11042: Ampicillin Resistance

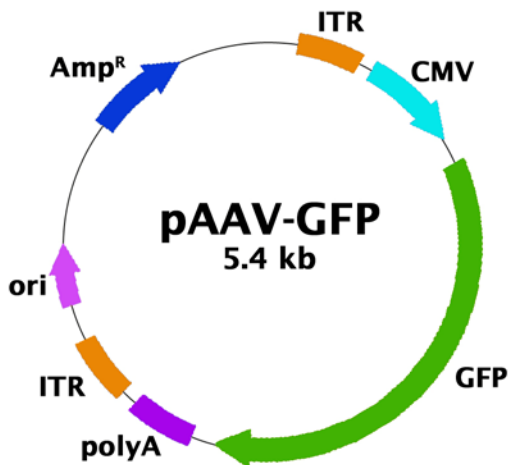


Figure 6. pAAV-GFP Vector

Vector Features:

- 1 ~ 130: Left ITR
- 139 ~ 801: CMV Promoter
- 809 ~ 1301: human β -globin intron
- 1324 ~ 2064: GFP
- 2123 ~ 2601: PolyA
- 2635 ~ 2775: Right ITR
- 3692 ~ 4552: Ampicillin Resistance

rAAV Production

1. One day before transfection, plate sufficient 293 cells or 293AAV cells (Cat. # AAV-100) to achieve 70-80% confluence on the day of transfection.
2. Cotransfect cells with pAAV Expression vector, pAAV-RC and pHHelper.

Notes:

- We recommend the ratio of vectors at 1:1:1 (pAAV Expression Vector:pAAV-RC:pHelper).
- Calcium Phosphate transfection method is preferred for AAV production. For lipid-based transfection reagents, we only suggest FuGENE® 6 (Roche Applied Science) or Lipofectamine™ LTX (Invitrogen).

3. 48-72 hours after transfection, add 0.5 M EDTA to a final of 10 mM to the plate and incubate for 3 min at room temperature. Gently shake the culture plate several times and harvest all media, including cells, in a sterile tube.

Notes:

- *As viral production proceeds, some of the cells will round up and detach from the plate, and can be seen as floating in the medium.*
 - *Viruses are present in both intact cells and the growth medium. For more concentrated virus stock, we only recommend proceeding with cell pellet.*
4. Centrifuge the cell suspension at 1000 RPM for 5 min. Remove the supernatant and resuspend the cell pellet in desired amount of DMEM or sterile PBS.
 5. Freeze and thaw the cell suspension four times by placing it alternately in a dry ice/ethanol bath and a water bath of 37°C. Remove cell debris by centrifugation at 10,000 g for 10 min and collect the supernatant as AAV crude lysate.
 6. AAV crude lysate can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80°C in aliquots.

Post-Packaging Considerations

The quality of rAAV vector preparations is a key element in obtaining reliable and reproducible data. Purification of rAAV from crude cell lysate is usually required before transduction of your target cells. rAAV is usually quantified by genome copy (GC) number. These genome-containing particles are functional vectors that infect target cells. Besides these "full" AAV, empty viral particles are also produced. Measurement of GC rather than total particle number is thus more relevant.

1. **Concentration and purification of your rAAV:** Recombinant AAV vector can be purified by CsCl gradient ultracentrifugation, iodixanol discontinuous gradient ultracentrifugation, and high-performance liquid chromatography (HPLC). The most popular technique, CsCl ultracentrifugation, is time consuming process which may result in poor recovery and quality (nonviral protein contamination and a high ratio of genome copies versus infectious units). For AAV-2, we recommend using Cell Biolabs' ViraBind™ AAV Purification Kit (Catalog # VPK-140).
2. **Measure titer of your rAAV:**
 - a. **Genome Copy (GC) Number:** This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or dot blot of viral DNA can take as much as 1-4 days to complete. An ELISA method has been developed by using antibody that only reacts with AAV intact particles; however, this method measures all AAV particles including the ones lacking genomic DNA. Cell Biolabs' QuickTiter™ AAV Quantitation Kit (Catalog # VPK-145) does not involve cell infection; instead it specifically measures the viral nucleic acid content of purified viruses or unpurified viral supernatant sample. The entire procedure takes about 4 hours for unpurified supernatant or about 30 minutes for purified AAV.
 - b. **Infectious Titer:** For AAV vector containing reporter, the rAAV infectious titer can be determined using either green fluorescent protein (GFP) or LacZ as the reporter gene. For rAAV-LacZ, each blue cell after X-Gal staining represents one infectious unit (IU). For rAAV-GFP, each green cell under fluorescence microscopy represents one IU.

3. **Use transduction reagents to increase infection efficiency:** The AAV transduction process includes viral binding and entry, intracellular trafficking, nuclear transport, and viral second strand DNA synthesis. The viral second strand DNA synthesis has been shown to be the rate limiting step, which leads to inefficient transduction by AAV vectors. Cell Biolabs' ViraDuctin™ AAV Transduction Kit (Catalog # AAV-200) is designed to increase transduction efficiencies by AAV on both dividing and non-dividing cells.

Example of Results

The following figure demonstrates typical results seen with Cell Biolabs' AAV Helper-free System. One should use the data below for reference only.

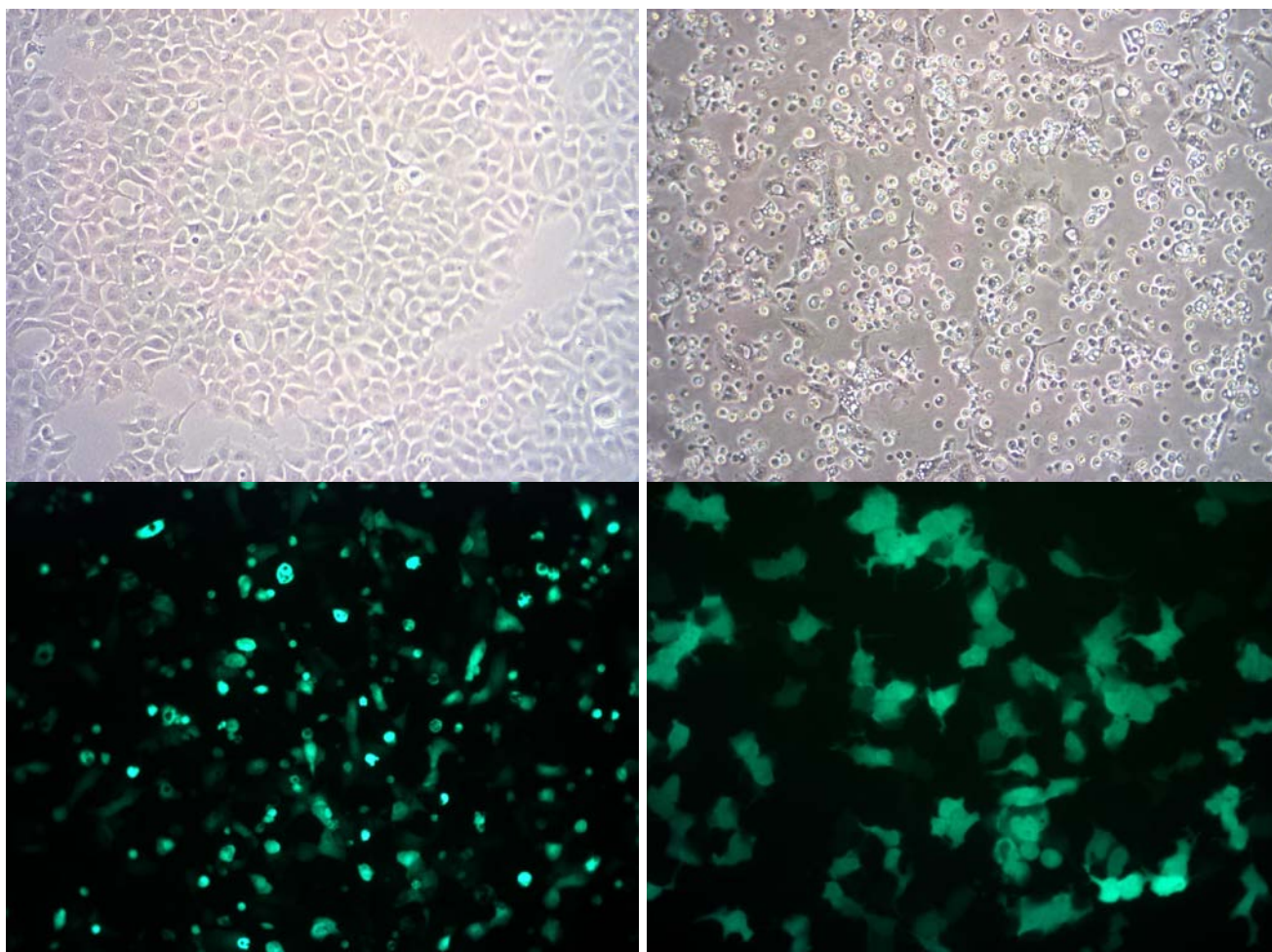


Figure 7: AAV2-GFP Production and Transduction: AAV2-GFP is produced by cotransfecting 293AAV cells (Cat.# AAV-100) with pAAV-GFP (Cat.# AAV-400), pAAV-RC2 and pHelper. 293AD Cells (Cat.# AD-100) were infected with AAV2-GFP viral supernatant for 48 hrs. **Top left:** 293AAV cells before transfection (10X); **Top right:** 293AAV cells 48 hrs after transfection (10X); **Bottom left:** GFP Expression in 293AAV cells 48 hrs after transfection (10X). **Bottom right:** GFP Expression in 293AD cells 48 hrs after transduction (20X).

References

1. Auricchio, A., Hildinger, M., O'Connor, E., Gao, G. P. and Wilson, J. M. (2001) *Hum Gene Ther* **12**:71–6.
2. Brument, N., Morenweiser, R., Blouin, V., Toublanc, E., Raimbaud, I. et al. (2002) *Mol Ther* **6**:678–86.
3. Clark, K., Liu, X., McGrath, J., and Johnson, P. (1999) *Hum. Gene Ther.*, **10**, 1031-1039.
4. Graham, F. L., Smiley, J., Russell, W. C. and Nairn, R. (1977) *J Gen Virol* **36**:59-74.
5. Grimm, D. and Kleinschmidt, J. A. (1999) *Hum Gene Ther* **10**:2445-50.
6. Matsushita, T., Elliger, S., Elliger, C., Podsakoff, G., Villarreal, L. et al. (1998) *Gene Ther* **5**:938-45.
7. McCarty, D. M., Monahan, P. E. and Sumulski, R. J. (2001) *Gene Therapy* **8**:1248-1254.
8. Rabinowitz, J, and Samulski, R. J. (1998) *Curr. Opin. Biotechnol.*, **9**, 470-475.
9. Russell, D. W., Alexander, I. E. and Miller, A. D. (1995) *Proc Natl Acad Sci U S A* **92**:5719-23.
10. Summer ford, C., and Samulski, R. J. (1999) *Nat. Med.*, **5**, 587-588.

Appendix

pAAV-IRES-GFP Plasmid Features and Sequence

1-130:	Left ITR
139-801:	CMV Promoter
809-1301:	Human β -globin Intron
1308-1376:	MCS
1407-1985:	IRES
1986-2705:	GFP
2717-3195:	PolyA
3235-3375:	Right ITR
4292-5152:	Ampicillin Resistance

```
CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGGCGACCTTTGGTCGCCC
GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTGCGGCC
GCACGCGTGGAGCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAG
TTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGAC
GTCAATAATGACGTATGTTCCCATAGTAACGTCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGT
ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGAC
GTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGT
GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGC
GTGGATAGCGGTTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTTGTTT
TGCACCAAATCAACGGGACTTTCCAAAATGTCGTAACAAC'TCCGCCCATTTGACGCAAATGGGCGGT
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTTAGTGAACCGTCAGATCGCCTGGAGACG
```

CCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGATTGGAATCCC
GGCCGGGAACGGTGCATTGGAACGCGGATTCCTCCCTGCAAGAGTGACGTAAGTACCGCCTATAGAGT
CTATAGGCCACAAAAAATGCTTTCTTTCTTTAATACTTTTTTTGTTTTATCTTATTTCTAATACTTT
CCCTAATCTCTTTCTTTTCAAGGCAATAATGATACAATGTATCATGCCTCTTTGCACCATTCTAAAGAA
TAACAGTGATAATTTCTGGGTAAAGGCAATAGCAATATTTCTGCATATAAATATTTCTGCATATAAAT
TGTAAC TGATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTACCATTCTGCTTTTATT
TTATGGTTGGGATAAGGCTGGATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGTTTCATAACC
TCTTATCTTCTCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAAG
AATTGGGATTCGAACATCGATTGAATTGGATCCGATATCTAGACAGAAGCTTGACCTCGAGCACGAAT
TCCTGCAGGCCTCGAGGGCCGGCGCCGCGGCGCTACGTAAATTCGCCCCCTCTCCCTAACGTTAC
TGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCACCATATTGCCGT
CTTTTGGCAATGTGAGGGCCCGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCCTAGGGGTCTTTCC
CCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTG
AAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCT
GCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGT
TGGATAGTTGTGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCA
GAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGA
GGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATGATAA
TATGGCCACAACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTTCGAGC
TGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC
AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCTGGCCACCCCTCGTGACCAC
CCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGT
CCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC
CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA
GGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGG
CCGACAAGCAGAAGAACGGCATCAAGGTGAACCTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG
CAGCTCGCCGACCCTACCAGCAGAACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCA
CTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGG
AGTTCGTGACCGCCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGTCGACGATCTACGG
GTGGCATCCCTGTGACCCCTCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCACCA
GCCTTGTCTAATAAAAATTAAGTTGCATCATTTTGTCTGACTAGGTGTCTTCTATAATATTATGGGG
TGGAGGGGGGTGGTATGGAGCAAGGGGCAAGTTGGGAAGACAACCTGTAGGGCCTGCGGGGTCTATTG
GGAACCAAGCTGGAGTGCAGTGGCACAATCTTGGCTCACTGCAATCTCCGCCTCCTGGGTTCAAGCGA
TTCTCCTGCCTCAGCCTCCCGAGTTGTTGGGATTCCAGGCATGCATGACCAGGCTCAGCTAATTTTTG
TTTTTTTTGGTAGAGACGGGGTTTTACCATATTGGCCAGGCTGGTCTCCAACCTCTAATCTCAGGTGAT
CTACCCACCTTGGCCTCCCAAATTGCTGGGATTACAGGCGTGAACCACTGCTCCCTTCCCTGTCTCTC
TGATTTTGTAGGTAACCACGTGCGGACCGAGCGGCCGAGGAACCCTAGTGATGGAGTTGGCCACTC
CCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTGCCCCGACGCCCGGGCTTTGCC
CGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGCGCCTGATGCGGTATTTTCTCCT
TACGCATCTGTGCGGTATTTACACCCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGC
ATTAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCG
CTCCTTTGCTTTTCTTCCCTTCTTTCTCGCCACGTTCCGCCGCTTTCCCGTCAAGCTCTAAATCGG
GGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCAAAAAACTTGATTTGGGTGA
TGTTTACAGTAGTGGGCCATCGCCCTGATAGACGGTTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT
TTAATAGTGGACTCTTGTTCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGAATTA
TAAGGGATTTTGCCGATTTGCGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAA
TTTTAACAAAATATTAACGTTTACAATTTTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCAT

AGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCC GGCA
TCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCCGTCATCACC
GAAACGCGGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGG
TTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAA
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAG
GAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTTTGCGGCATTTTGCCTTCCTG
TTTTTGTCTACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGT
TACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCGAAGAACGTTTTCCAAT
GATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCCTATTGACGCCGGGCAAGAGCAAC
TCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTT
ACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAA
CTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATG
TAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATAACAAACGACGAGCGTGACACCAG
ATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGGCGAACTACTTACTCTAGCTTCCCG
GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGG
CTGGCTGGTTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTG
GGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGA
ACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTT
ACTCATATATACTTTAGATTGATTTAAAACCTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTT
TTTGATAATCTCATGACCAAAATCCCTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGA
AAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAC
CACCGCTACCAGCGGTGGTTTTGTTTGC CGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGC
TTCAGCAGAGCGCAGATACCAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAA
CTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATA
AGTCGTGTCTTACCGGGTTGACTCAAGACGATAGTTACC GGATAAGGCGCAGCGGTGCGGCTGAACG
GGGGTTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA
GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTTCG
GAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCTCTGTCGGGTTT
CGCCACCTCTGACTTGAGCGTCGATTTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGAAAAACGC
CAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGTGCTGGCCTTTTGTCTCACATGT

Notice to Purchaser

Cell Biolabs, Inc. agrees to sell, and Customer agrees to purchase Cell Biolabs' AAV vectors provided herewith (referred to as the "Products") on the following terms and conditions. (For purposes of this Notice, "Customer" shall include any person or entity which ordered the Products or at any time uses the Products). Customer's acceptance of delivery and/or use of the Products shall constitute Customer's binding agreement to the following terms and conditions. If Customer is unwilling to accept such terms and conditions, Customer must return the Products prior to any use of the Products and shall be entitled to a full refund.

1. The Products provided herewith are covered by issued U.S. and/or foreign patents and/or pending U.S. and foreign patent applications owned by Genzyme Corporation ("Licensor"). Cell Biolabs has the right to sell the Products for use by Customer for internal *in vitro* or *in vivo* research purposes only, wherein said right specifically excludes, without limitation, (i) any use of Products and/or materials made using Products in humans and (ii) any transfer of Product or materials made using

Products to a third party. No other rights are conveyed with the sale of Products hereunder. Purchase of the Products does not convey any rights to modify the Products, offer the Products or any derivatives thereof for resale, or distribute or transfer the Products or any derivatives thereof to any third parties.

2. The Products shall be used solely on the premises of and under the control of Customer, and in compliance with all laws, regulations, rules and guidelines applicable to the Products and their use, testing, handling, or other dispositions thereof, or otherwise applicable to Customer's activities hereunder.

3. THE PRODUCTS ARE EXPERIMENTAL IN NATURE AND IS PROVIDED WITHOUT WARRANTIES OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Customer hereby waives, releases and renounces any and all warranties, guarantees, obligations, liabilities, rights and remedies, express or implied, arising by law or otherwise, with respect to the usefulness or freedom from defects of the Products, including, but not limited to, (a) any implied warranty of merchantability or fitness for a particular purpose, (b) any implied warranty arising from course of performance, course of dealing or usage in the trade, and (c) any claim or remedy for (1) loss of use, revenue or profit, or any other damages, (2) infringement of third party intangible property rights, and (3) incidental or consequential damages.

4. Customer agrees to bear all risks associated with the Products and their use, testing, handling or other disposition thereof. Customer hereby assumes all risks of damage or injury to Customer's facilities, employees or agents and to any third party arising from possession or use of the Products. Genzyme Corporation shall have no liability to Customer, its employees or agents or to any third party, regardless of the form or theory of action (whether contract, tort or otherwise, including but not limited to, negligence and strict liability), for any direct, indirect, consequential, incidental or other damages arising out of or relating to the Products or this Agreement.

5. Customer shall indemnify, defend and hold Cell Biolabs, Genzyme, and their licensors, affiliates, distributors, suppliers, directors, officers, employees and agents, harmless from and against any and all claims, actions, demands, liabilities, damages and expenses (including attorneys' fees) relating to or arising out of any damage or injury, including, but not limited to, personal injury and death, alleged to have been caused by the Products or the use, testing, handling or other disposition thereof or Customer's activities hereunder.

6. Customer may at any time properly dispose of the Products in a manner which ensures their prompt destruction and is consistent with all applicable laws, regulations, rules and guidelines.

7. No modification or waiver of any terms or conditions of this Notice shall be effective unless in a writing signed by Customer and an authorized representative of Genzyme. For information on purchasing a license to use the Products for non-research purposes, including commercial manufacturing, clinical manufacturing, commercial sale, or use in clinical trials, please contact: Sr. Vice President of Corporate Development, Genzyme Corporation, 500 Kendall Street, Cambridge, MA 02142.

8. Customer acknowledges and agrees that Genzyme Corporation is an intended third-party beneficiary of this Notice, with the right to enforce the foregoing restrictions.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2010-2016: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.