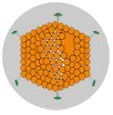
Recombinant Adeno-Associated Virus (rAAV)

Instruction Manual

BraInVTA

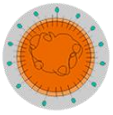
VECTOR FOR LIFE

Plasmid Vectors



AAV

LV



Other Virus

Content

Disclaimer

BrainVTA only offers virus packaging services, and we shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Although provided in a highly purified form, our products are not intended for clinical diagnosis or drug use. They are for research purposes only.

Ordering Information

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Safety And Storage

Safety

Recombinant AAV constructs do not encode for either a potentially tumorigenic gene product or a toxic molecule. According to guidelines from the National Institutes of Health (NIH), recombinant AAV vectors can be handled in a Biosafety Level1 (BSL-1) environment. Please refer to corresponding instructions if dealing with biohazardous materials.

Storage

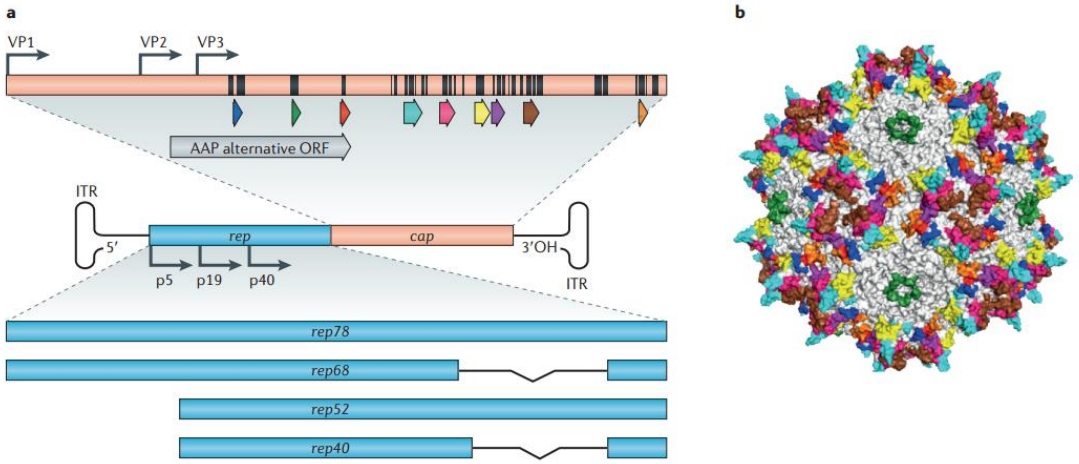
AAV stocks are supplied in liquid form and the storage solution is phosphate-buffered saline(PBS) with 0.001% Pluronic F68 (F68). For short-term storage, all vectors could be stored at room temperature or 4°C. For long-term storage, keep all vectors at -80°C. Do NOT store at -20°C. We recommend your virus be aliquoted into desired volumes and stored at -80°C immediately. Please avoid unnecessary freeze/thaw cycles of viruses, which may result in a significant decrease in titer and biological activity.

Introduction

Adeno-associated virus (AAV) was discovered in 1965, as a contaminant of adenovirus (Ad) preparations, hence the name. It is a replication-defective, non-enveloped small virus (20nm) from the parvovirus family with a genome of a single stranded DNA. AAVs have been tested positive among 80-90% of humans without causing any malicious disease.. Recombinant AAVs can infect both dividing and non-dividing cells and persist in an extrachromosomal state without integration into the genome of the host cell. Those features make rAAVs ideal viral vectors for gene therapy.

AAV Genome Structure

AAV has a linear single-stranded DNA (ssDNA) genome of approximately 4.7kb. The genome comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs): Rep and Cap. The Rep is composed of four overlapping genes encoding Rep proteins required for the AAV life cycle, and the Cap contains overlapping nucleotide sequences for capsid proteins: VP1, VP2 and VP3, which interact together to form a capsid of a symmetry icosahedron in a ratio of 1:1:10.



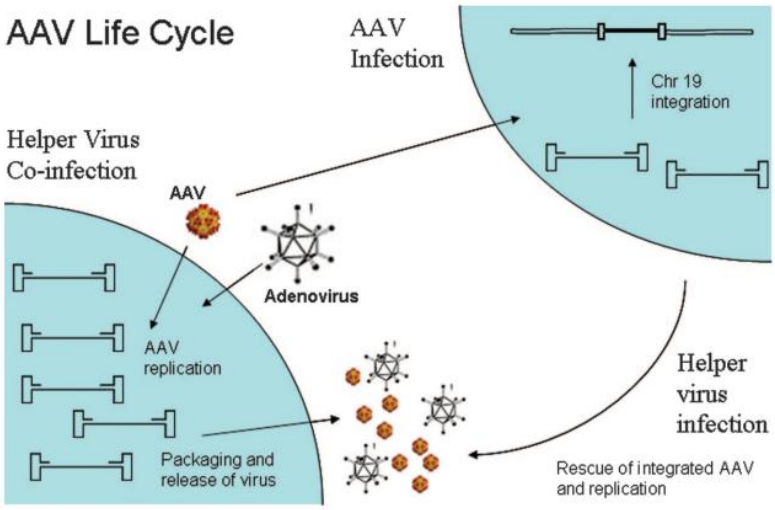
Adeno-associated virus biology. a: AAV Genome Structure b: Crystal structure of the AAV capsid（Melissa A. Kotterman et al. Nature Reviews Genetics.2014.）

AAV Life Cycle

In the presence of helper virus (adenovirus or herpesvirus), AAV undergoes productive transduction characterized by genome replication, viral gene expression, and virion production. The life cycle includes:

(1) Combine with receptor;
(2) Receptor-mediated endocytosis;
(3) Endosomal trafficking;
(4) Escape from the late endosome or lysosome;
(5) Translocation to the nucleus;
(6) Uncoating;

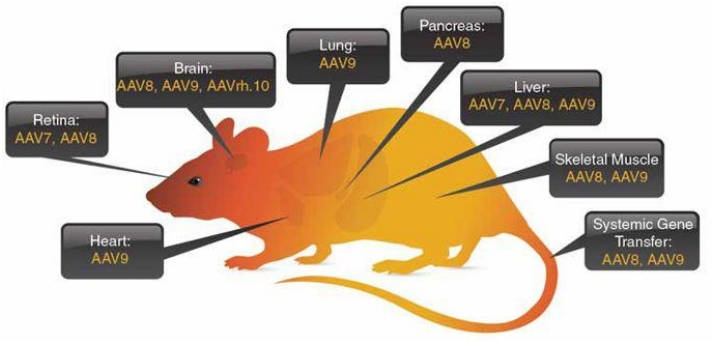
(7) DNA replication;
(8) Assembly of complete virions, and release from the transduced cells.
In the absence of helper virus, the AAV genome can establish latency by integrating into a 4kb region on chromosome 19 (q13.4), termed AAVS1.



AAV life cycle. (Shyam Daya et al.Clinical Microbiology Reviews.2008)

AAV serotypes and native tropism

So far, 12 AAV natural serotypes have been isolated, they all have different tropism and can infect cells from multiple diverse tissue types. Among them, AAV2, AAV3, AAV5 and AAV6 have been discovered from human cells, AAV serotypes 1, 4, and 7-11 in nonhuman primate samples. Different AAV serotypes bind to different cell receptors lead to the tissue specificity.



Karen Kozarsky et al.Nature Methods.2010.

Viral Vector Selection Guide

With the development of viral gene delivery systems, many different viruses are being adapted as vectors to introduce genes into diverse types of cells and tissues, with lentivirus (LV), adenovirus (Ad) and adeno-associated virus (AAV) being the most advanced. When designing your experiment, you'll need to consider which vector to choose. Here are some key factors to consider:

● **Gene expression.**

Do you want transient or stable gene expression in cells ?

● **Transduction.**

Do you need to transduce dividing or non-dividing cells?

● **Immune response.**

How important is potential immune response for your target cell?

● **Size.**

How large is the gene of interest?

The table below summarizes the main characteristics of different vector systems.

viral vector	AAV	LV	Ad
Genome	ssDNA	ssRNA (+)	dsDNA
Coat	Naked	Enveloped	Naked
Type	Non-integrating	Integrating	Non-integrating
Transduction	Dividing and non-dividing cells	Dividing and non-dividing cells	Dividing and non-dividing cells
Packaging Capacity	4.5kb	6.5kb	7.5kb
Transgene Expression	Potentially long-lasting	Long-lasting	Transient
Immune Response	Very Low	Low	High

In short, AAVs are suitable for genes less than 4.5 kb plus ITRs and long-lasting expression in non-dividing cells as well as transient expression in dividing cells. LV vectors are often used for gene integration. Adenovirus is useful in transient expression of a medium-sized gene.

AAV Related Services

BrainVTA offers vector cloning and packaging services, including gene expression, RNAi and gene editing. All processes use our in-house optimized methods to generate high quality viruses. The charts below show most commonly used promoters and serotypes. Novel serotypes or promoters can also be produced on a case-by-case basis.

Most Commonly Used Promoters

Ubiquitous potent promoters		
Name	**Characteristics**	**Size (bp)**
CMV	Ubiquitous	584
CAG	Ubiquitous	1678
nEf1α	Short Ef1α, Ubiquitous	493
Ef1α	Ubiquitous	1179
hUbC	Ubiquitous	1130
Inducible promoters		

Name	Characteristics	Size（bp）
TRE3G	Tetracycline(Tet)-inducible	350
Activity–dependent promoters		
Name	**Characteristics**	**Size（bp）**
C-fos	Immediate-early gene promoter	662
E-SARE	Enhanced synaptic activity–responsive element	954
Neuron-specific promoters		
Name	**Characteristics**	**Size（bp）**
Mecp2	Truncated Mcep2 neuron specific	230
hSyn	Mature neuron specific	485
CaMKIIa	Specific expression in excitatory neurons in the neocortex and hippocampus	1293
PV	GABAergic neuron subtypes	2396
VGAT	GABAergic neuron	1800
mDlX	GABAergic neuron	530
TH	Dopaminergic neuron specific	299
TPH2	Tryptophan hydroxylase promoter	2042
ChAT	Cholinergic neuron specific	1500
GFAP	Astrocyte specific	2207
Cx30（GJB6)	Astrocyte specific	1505
CX3CR1	Microglia specific	1500
Mash1/Ascl1	Neural stem cell specific	1275
L7/Pcp2	Purkinje cell	990
MBP	Myelin basic protein promoter, efficient transduction of oligodendrocytes	1300
TRPV1	Transient receptor potential cation channel, subfamily V, member 1	2073
TRPV2	Transient receptor potential cation channel, subfamily V, member 2	1700
D1	D1 dopamine receptor	827
D2	D2 dopamine receptor	1211
OT	Oxytocin promoter	2612
CRH	Corticotropin-Releasing Hormone (CRH) promoter	1600
PTH	Human parathyroid hormone promoter	925
Eye-specific promoters		
Name	**Characteristics**	**Size（bp）**
hGRK1	Human rhodopsin kinase promoter	292
CAR	Cone arrestin gene promoter	523
mRHOP	Rhodopsin gene promoter	524

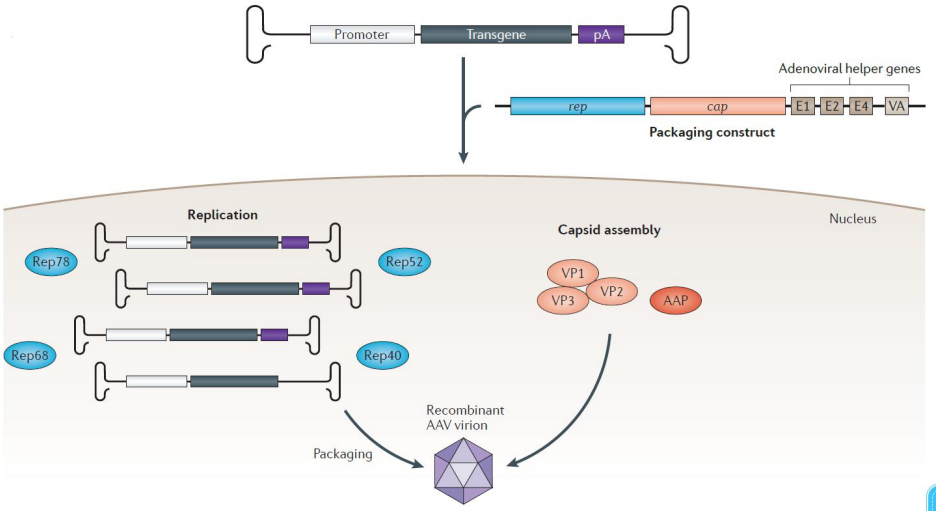
Nrl	Neural retina leucine zipper gene promoter	3200
RK	Rhodopsin kinase promoter	295
Liver-specific promoters		
Name	**Characteristics**	**Size（bp）**
ALB	Albumin enhancer promoter	2400
TBG	Serine (or cysteine) peptidase inhibitor, clade A	410
Cardiac-specific promoters		
Name	**Characteristics**	**Size（bp）**
cTNT	Cardiomyocytes-specific promoter	734
CS-CRM4 -αMHC	Cardiomyocytes-specific promoter	590
Muscle-specific promoters		
Name	**Characteristics**	**Size（bp）**
MCK	Muscle creatine kinase (MCK) promoter	1358
dMCK	MCK promoter by ligating a double tandem of MCK enhancer	509
tMCK	MCK promoter by ligating a triple tandem of MCK enhancer	720

The pre-selected serotypes

Sserotype	Tropism
AAV1	Muscle, heart, CNS, eye, lung, skeletal muscle
AAV2	In vitro, CNS, eye, muscle, live, brain
AAV5	Lung , eye, CNS, pancreas, adipose, liver
AAV6	Muscle, lung, heart
AAV8	Liver, muscle, eye, CNS, adipose, pancreas
AAV-pan	Pancreas
AAV9	Lung , liver, muscle, heart, CNS, adipose, BBB
AAV.rh10	CNS, BBB, pleura,
AAV-Rec2	Adipose
AAV.DJ	In vitro, liver, heart, kidney
AAV2/Retro	Retro
AAV.PHP.S	DRG, heart, colon
AAV.PHP.B	BBB
AAV.PHP.eB	BBB

AAV virus packaging

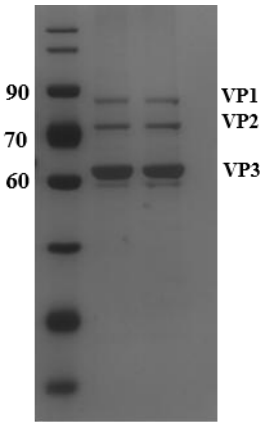
After subclone the gene of interest (GOI) into a related pAAV cis-plasmid, transfections are performed together with a plasmid encoding rep and serotype-specific cap, and a plasmid encoding helper sequences. AAV viruses are produced from HEK 293 cells and are purified by ultracentrifugation on an iodixanol step gradient.



AAV virus packaging. (Melissa A. Kotterman.Nature Reviews et al.2014.)

Purification

Viral particles are purified by ultracentrifugation on an iodixanol step gradient and the purity of AAV is assayed by comparing the components of the capsid proteins VP1, VP2 and VP3. The molecular weight and relative intensity of VP1, VP2 and VP3 are analyzed by SDA-PAGE. The image below shows the protein components in our purified AAV virus.

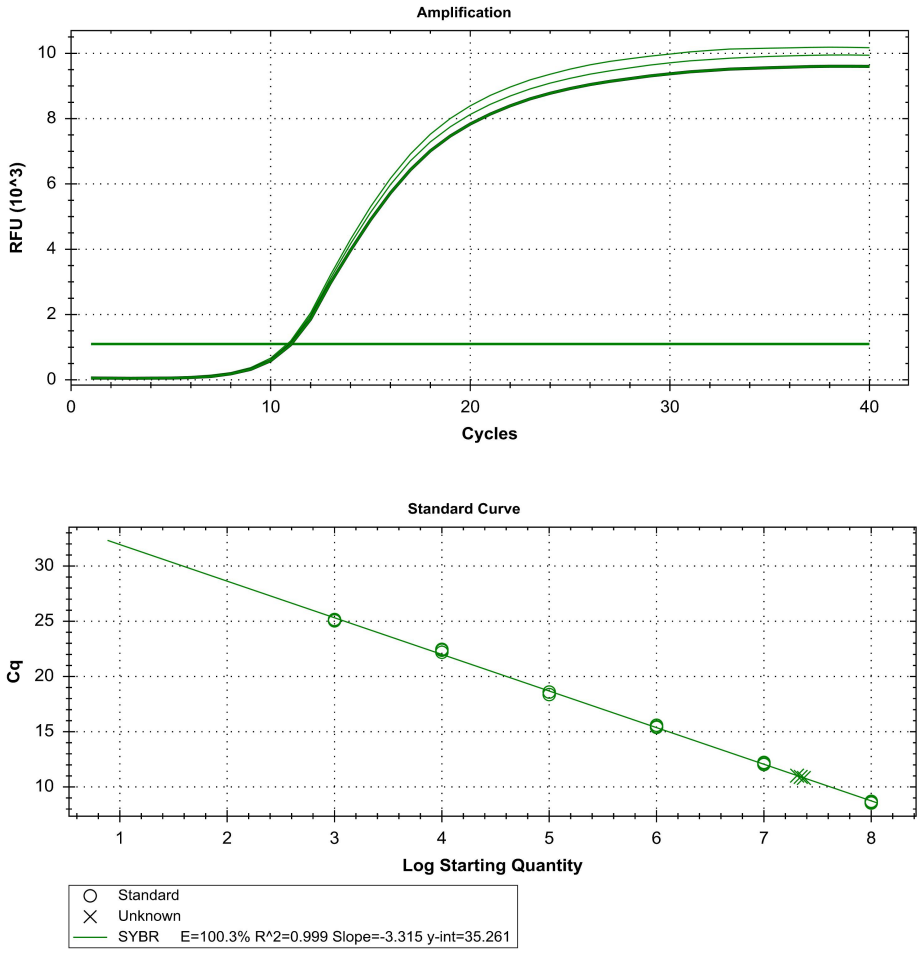


Purity of purified AAV virus

Titration

The virus titer is determined by the viral genome copy number by real-time quantitative PCR using primers targeting the inserted DNA sequences such as *wpre, cmv and GFP* instead of ITRs to avoid false high titers.

Following example is data for tittering AAV-GFP virus.



The virus titer of rAAV-GFP is 10^{12}vg/mL.

Recommended Protocol For Use In Vitro And In Vivo

In vitro cell transduction

The efficiency of AAV transduction is dependent on cell types. The chart shows the relative efficiency of transduction of AAV vectors in vitro. Transductions are performed use the minimum concentration of FBS and you should look for expression at 24h, 48h, 72h and 96h, post transduction.

Cell Line	AAV-1	AAV-2	AAV-3	AAV-4	AAV-5	AAV-6	AAV-8	AAV-9	AAV-DJ	AAV-DJ/8
Huh-7	13	100	2.5	0.0	0.1	10	0.7	0.0	500	0.2
HEK293	25	100	2.5	0.1	0.1	5	0.7	0.1	500	0.3
HeLa	3	100	2.0	0.1	6.7	1	0.2	0.1	667	0.2
HepG2	3	100	16.7	0.3	1.7	5	0.3	ND	1250	0.5
Hep1A	20	100	0.2	1.0	0.1	1	0.2	0.0	400	0.1
911	17	100	11	0.2	0.1	17	0.1	ND	500	0.0

CHO	100	100	14	1.4	333	50	10	1.0	25000	5.0
COS	33	100	33	3.3	5.0	14	2.0	0.5	500	0.3
MeWo	10	100	20	0.3	6.7	10	1.0	0.2	2857	1.0
NIH3T3	10	100	2.9	2.9	0.3	10	0.3	ND	500	0.1
A549	14	100	20	ND	0.5	10	0.5	0.1	1000	0.1
HT1180	20	100	10	0.1	0.3	33	0.5	0.1	333	0.2
Monocytes	1111	100	ND	ND	125	1429	ND	ND	100	ND
Immature DC	2500	100	ND	ND	222	2857	ND	ND	200	ND
Mature DC	2222	100	ND	ND	333	3333	ND	ND	100	ND

Note: Efficiency normalized to AAV-2 = 100. ND = Not Determined.

1. Grimm, D. et al. (2008). *J. Virol.* **82**: 5887-5911.

In vivo animal use

- The recommended dosage of AAV for CNS injection is about 10^{12}vg/ml in a 300 nL volume.
- The recommended dosage for peripheral injection is 10^{11} vg (body weight).
- Dilute the virus with PBS to achieve appropriate titer.

FAQ

How are viral titers determined?

Titers are determined for each vector type by means of a combination of methods to assess the total number of particles (both live and dead/empty) or the total number of live “infectious” particles. AAV titers are given as a “physical” titer in viral genomes per ml (vg/ml) determined by direct qPCR of purified vector particles. Vectors are also assessed for transduced titer when fluorescent reporters are available. All AAV titers as well as capsid proteins are further verified by Silver Stain.

Which control AAV should I use?

The primary factors to take into consideration when selecting the control AAV are serotypes, promoters, and reporters. Ideally, you control AAV should be the same serotype AAV expressing a reporter driven by the same promoter as the AAV expressing your GOI.

How far in advance do you have to schedule an AAV production project?

Our production timeline is 5-8 weeks for custom projects. The delivery times provided are estimates only and cannot be guaranteed. Delays may occur due to unforeseen difficulties inherent to the nature of cloning work. We take customer orders daily and will answer your questions through: **sales@brainvta.com** .

How much plasmid do I need to send?

We need at least 10 μg of plasmid if we start from cloning. If you provide in a smaller amount, we will spend additional time on DNA amplification during the cloning step. The DNA does not have to be prepared with an endotoxin free kit.

How do you pack and ship your products?

We pack our products tubes in a bubble mailer or bubble wrap in a Styrofoam container with or without an outer cardboard box. Parcels will be shipped through third-party couriers on either ice packs (4°C) or dry ice (-80°C) for a flat rate that is adjusted for distance shipped.

What should I do after I get the product?

Upon receiving, vectors should be stored at -80℃.

Can I freeze and thaw the virus?

We have tested the stability of vectors through multiple freeze-thaw cycles and find that the titer is consistent for approximately 3 freeze-thaw cycles before it begins to drop; however, we recommend that investigators limit freeze/thaw cycles to as few as possible. Vectors are stable for a short period of 24-48 hours at 4℃. We recommend that the vector be stored at -80℃ upon receipt. Thaw the vector only when you are ready to use it and make aliquots of no less than 25 μl in 0.5 ml tubes.

How long does it take for my package to arrive once it has been shipped out?

Country	Transit Time (Days)
US & Canada	2 to 3 days
Europe	2 to 3 days
Asia	3 to 5 days

Do you have a local distributor in my country?

Yes! We have a local distributor in the US currently.

I'm a bio-reagent wholesaler/distributor, can I order your product for my customer?

Yes. Please contact us for conditions and limitations for distributors.

My institute requires a quote before I can place the order, how can I get one?

Just email us your name, name of your institution, telephone number, and the catalog number and quantity for each product you want to order, and we will email you an official quote in PDF file.

Shipping, Delivery, and Packaging Fees?

All shipping & packaging fees will be included in the quote, prepaid and added to the invoice. To reduce packaging costs, environmental impact, or materials used, we will always pack shipments together if they will be sent to the same address within 2-3 days.