FAQs

Using Adeno-Associated Virus for Gene Delivery

General questions

What are the main features of adeno-associated viruses?

Adeno-associated virus (AAV) is a non-enveloped virus that belongs to the *Parvovirus* family of the *Dependovirus* genus. AAV is not thought to be pathogenic to humans and only replicates in the presence of a helper virus, such as adenovirus or herpesvirus. The capsid structural proteins determine the ability of the virus to infect tissues/cells; tissue-specificity is known to differ for various serotypes.

What features of AAV makes the virus a good vector for gene delivery?

AAV vectors exploit the properties of AAV for transduction of genes to cells and organisms. AAV vectors are generally considered safer than adenoviral and retroviral vectors. AAV vectors can be used to transduce genes into both proliferating and non-proliferating cells and can impart long-term expression in non-dividing cells. In addition, AAV has little immunogenicity and is suitable for the transduction of genes into animals (as an *in vivo* transduction tool).

What are the features of different AAV serotypes?

Serotype is classified by the type of capsid structural proteins and determines tissue specificity. Serotype 2 (AAV2) is the most well-studied serotype. AAV2 has a broad range of tissue infectivity and is the most popular serotype for research purposes. Serotype 5 (AAV5) and serotype 6 (AAV6) have more specific tissue infectivity; AAV5 can infect cells in tissues including the central nervous system, liver, and retina, whereas AAV6 can infect cells in tissues including heart, muscle, and liver.

We currently offer products for production, purification, and titration of AAV2 for gene delivery.

Learn more >>

What precautions should be taken when working with recombinant AAV?

It is imperative to fully understand the potential hazards of and necessary precautions for laboratory use of AAV vectors. Viruses could, depending on your gene insert, be potentially hazardous. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant viruses.

Follow all applicable guidelines for research involving recombinant DNA. Take appropriate safety measures when producing or handling recombinant AAV, including working in a biological safety cabinet and wearing protective laboratory coats, face protection, and gloves.

Are there published examples of AAV2 being used for *in vivo* gene transfer?

AAV2 particles generated using the AAVpro series were successfully used for *in vivo* delivery of a bright fluorescent marker to cells in a very specific brain region in mouse.

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In addition, AAV2 was used for gene delivery in the following studies (none of these examples use AAVpro products).

Target tissue	Description	Citation
Brain	AAV2 used to deliver Cre to the subventricular zone	Lee N., *et al*. (2013) Ciliary neurotrophic factor receptor regulation of adult forebrain neurogenesis. *J. Neurosci.* **33**(3):1241–1258.
Brain	AAV2 was used for gene delivery directly to the hippocampus	Passini MA, *et al*. (2005) AAV vector-mediated correction of brain pathology in a mouse model of Niemann-Pick A disease. *Mol. Ther.* **11**(5):754–762.
Liver	AAV2 carrying a gene under the control of a liver-specific promoter was intravenously administered to mice	Ziegler RJ, *et al*. (2004) AAV2 Vector Harboring a Liver-Restricted Promoter Facilitates Sustained Expression of Therapeutic Levels of a-Galactosidase A and the Induction of Immune Tolerance in Fabry Mice. *Mol. Ther.***9**(2):231–240.
Pancreas	AAV2 was used for gene delivery to mouse pancreas	Wang AY, *et al*. (2004) Comparison of adenoviral and adeno-associated viral vectors for pancreatic gene delivery *in vivo*. *Hum Gene Ther.* **15**(4):405–413.

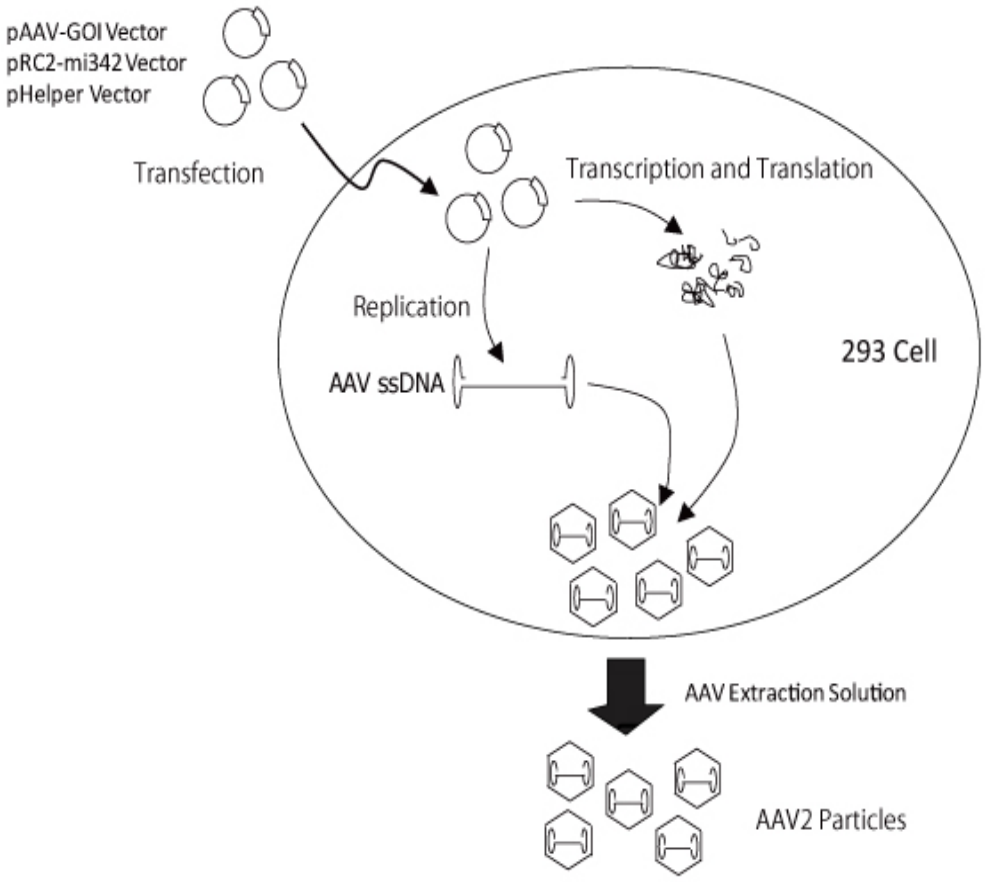
How much AAV should be used for general animal experiments?

The amount used depends on the target tissue and method of administration, but for mice the amount will be between 10^{11} and 10^{12} vg/animal (vg = vector genome, as measured by real-time PCR). However, the exact dose should be determined empirically.

Recombinant AAV production questions

What is “helper-free” production?

In general, replication of AAV requires a helper virus such as adenovirus or herpesvirus. The AAVpro Helper Free System generates recombinant AAV without the use of a helper virus (“helper-free”). Instead packaging is accomplished by introducing plasmids that encode the factors necessary to prepare recombinant AAV particles to HEK 293 cells by transfection.



What cells are suitable for the production of AAV particles?

For the AAVpro Helper Free System, use HEK 293T or HEK 293 cells.

Several HEK 293 and HEK 293T cell lines are commercially available. Viral production is highly dependent on features of the cell line. The Lenti-X 293T Cell Line and HEK 293T/17 cells (ATCC, CRL-11268) are recommended for preparation of high-titer AAV.

Infection of cultured cells cannot be confirmed; what could be the cause?

It is possible that high titers of AAV were not produced. Consider the following possible causes:

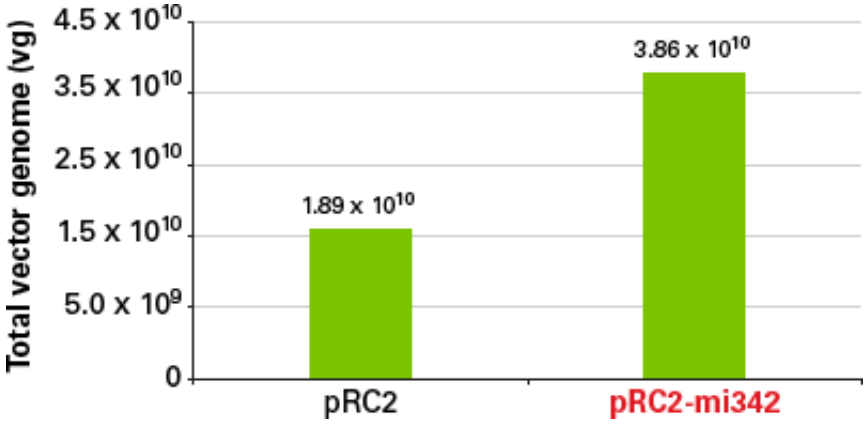
1. The HEK 293 cell line used may affect AAV production. We recommend using the Lenti-X 293T Cell Line or HEK 293T/17 (ATCC CRL 11268) cells. We have confirmed efficient AAV production for these cell lines.
2. It is possible that transfection efficiency is low. Be sure to follow the transfection methods outlined in the user manual.

Do you offer control vectors?

Yes, we have AAV vectors that express LacZ or ZsGreen1.

- What are the advantages of the AAVpro Helper Free System in comparison with AAV production kits from other companies?

Our kit includes a vector that expresses hsa-miR342, a human microRNA that increases AAV2 production (patent pending). This system increases titer by ~2-fold compared with the pRC2 Vector alone.



AAV purification questions

- Can the AAVpro Purification Kit be used for purification of AAV serotypes other than 2?

This kit is specific for serotype 2 (AAV2). Other serotypes can be purified by methods such as ultracentrifugation.

- What are the benefits of using the AAVpro Purification Kit?

Ultracentrifugation is commonly used for AAV purification. However, this technique requires a high skill level and several days (2–3) to complete. The AAVpro Purification Kit provides a simple and fast (~4 hours) column-based method for AAV2 particle purification from virus-producing cells.

Watch the video protocol >>

- What number of cells can be used for AAV purification with this kit?

The Prepacked Column in the AAVpro Purification Kit enables purification of AAV2 particles from up to ten T225 cell culture flasks.

- For administration to animals, how should AAV particles be purified?

There are no definitive purity criteria for *in vivo* transduction. You can confirm AAV purity by SDS-PAGE analysis; there should only be three major bands (corresponding to AAV VP1, VP2, VP3).
AAV2 particles purified using AAVpro Purification Kit (AAV2) have been used successfully for *in vivo* transduction in mice.

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- If the column flow rate becomes slow, what is the cause?

If there are air bubbles in the resin portion of the column, the flow rate may slow. Remove any air from the column with a pipette before use.

In addition, flow rate may be slow if the sample contains a large amount of impurities. You can remove impurities by filtration with a low adsorption 0.45-µm filter prior to purification.

AAV titration questions

- Which serotypes can be measured with the AAVpro Titration Kit (for Real Time PCR) Ver.2?

The primers in this kit are specific for the inverted terminal repeat (ITR) region of AAV2. Therefore, if AAV vector being used includes the ITR region from AAV2, it is possible to measure regardless of the serotype.

- What are the advantages of the AAVpro kit in comparison to other conventional titration methods?

Conventionally, a region of the inserted sequence is used for quantification of AAV vector genome. With this method, it is necessary to design primers for each AAV vector. The AAVpro Titration Kit (for Real Time PCR) Ver.2 uses a common region of the AAV vector, the AAV2 inverted terminal repeat (ITR), as a target. If the ITR region of the vector is derived from AAV2 (as is the case for most commonly used constructs), the kit can be used regardless of the serotype.

Learn more about AAVpro Titration Kit (for Real Time PCR) Ver.2 >>

AAV extraction questions

- Is AAVpro Extraction Solution compatible with any AAV serotype?

It can be used regardless of the serotype. However, for some serotypes lower extraction efficiency is obtained as compared with traditional methods. For AAV5, we have found lower extraction efficiency with AAVpro Extraction Solution as compared with freeze-thaw methods, **but** the final viral solution had less contaminants when AAVpro Extraction Solution was used.

Learn more about AAVpro Extraction Solution >>

- Upon addition of AAV Extraction Solution B, the solution turned pink; is this a problem?

Depending on the sample, the solution may turn pink upon addition of AAV Extraction Solution B, but this color change does not affect extraction efficiency.

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