GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Fax: 1 (800) 380-4198
Email: orders@gentarget.com

Pre-made recombinant Aeno-Association Virus(rAAV)

(For research use only. Not intended for any clinical use)

CAT#	Product Content
AAV#.#	Ready-to-use AAV (research grade, concentrated, non-purified) **100ul** x (**1x10^{12} GC/ml**)

Amount: 100ul/vial with the titer of **1 x 10^{12}** GC/ml (equivalent to 2x10^{12} VP/ml)
Storage: -80 °C, avoid repeat freeze/thaw cycles, stable for 12 months.

Notes:

1) rAAV stocks are supplied in PBS solution. To use it, thaw it at room-temperature or on ice, gently mix it using pipet. It may appear cloudy, which is normal.
2) Bio-safety Level is BSL-1 (or BSL-2 when carries potentially hazardous genes).
3) AAV titer was measured by qPCR. The titer is used as reference because of the deviation of qPCR assay.

1. Product Description,

1) About recombinant Adeno-Associated Virus (rAAV)

Adeno-Associated Virus (AAV) is a widely used viral vector system for delivering genetic material into various cell types, both in vitro and in vivo. AAV is a small (~25nm), non-enveloped virus belonging to the Parvovirus family. It is replication-deficient and requires helper viruses, such as Adenovirus or Herpes Simplex Virus, to replicate in its wild-type form.

The recombinant form of AAV (rAAV) has been engineered to remove all viral genes, leaving only the necessary inverted terminal repeats (ITRs) to enable packaging and genome stability. The Helper-free AAV system use three plasmids: transgene, pAAV-RC and Helper, to package the rAAV. The transgene plasmid does not share any regions of homology with the rep/cap-gene containing plasmid (pAAV-RC), preventing the production of wild-type AAV through recombination. This modification makes rAAV a safe and efficient tool for gene delivery in a wide range of research and therapeutic applications.

AAVs transduce a wide variety of mammalian cell types. They barely integrate into the host genome, reducing the risk of insertional mutagenesis. They are

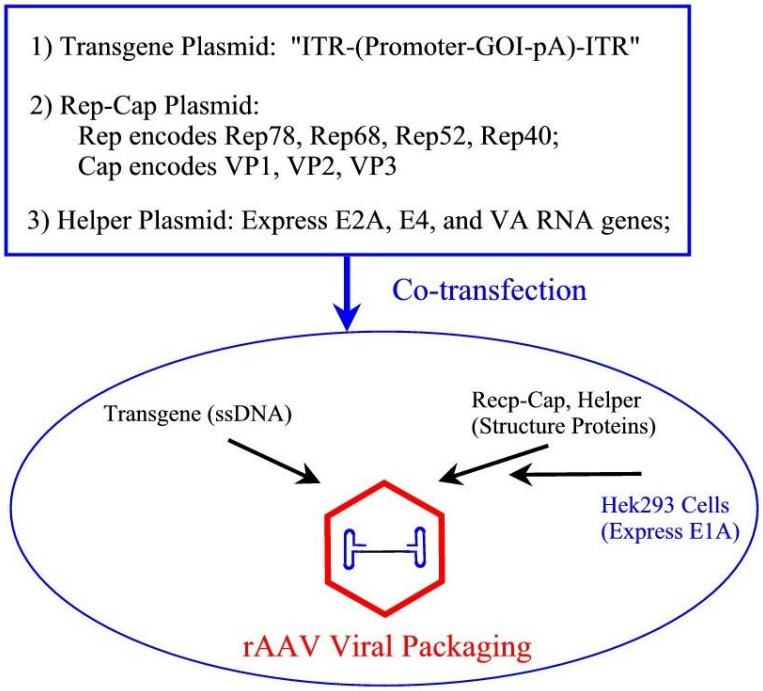
7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Fax: 1 (800) 380-4198
Email: orders@gentarget.com

non-pathogenicity and low immunogenicity in human, which make them a great tool for gene therapy. Click to see *FAQ about AAV*.

2) Gentarget Inc's Pre-made rAAV)

Gentarget's premade Recombinant Adeno-Associated Virus (rAAV) are packaged in HEK293T cells using three plasmids: the transgene plasmid, the helper-plasmid and the Rep/Cap plasmid.

The transgene-plasmid carries the gene of interest flanked by AAV Inverted Terminal Repeats (ITRs), which are essential for AAV genome packaging. It does not contain any viral genes, making rAAV non-replicative. The Rep/Cap-plasmid provides the Rep (Replication) and Cap (Capsid) genes, which are necessary for AAV replication and capsid formation. Gentarget rAAV uses AAV2's ITRs and Rep2 because AAV2 is the best characterized and full compatible with other serotype's Cap gene. Different Cap genes define the AAV serotype, determining the tropism of the virus. Gentarget coupled Rep2 with 10 Cap gene as AAV1 to AAV10 and DJ. The Helper-plasmid provides the packaging components: E2A, E4, and VA RNA, which enable AAV packaging without need live Adenovirus infection. The HEK293T cell express the necessary E1 for the packaging. See the packaging scheme below.



7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Fax: 1 (800) 380-4198
Email: orders@gentarget.com

With its proprietary technology in AAV transfer plasmid construction, ITRs integrity during plasmid prep, and in AAV virus packaging procedure, Gentarget is able to produce high titer AAV products for different targets, reporters, CAR-T, TCRs, knockout gRNA, knock-down shRNA, miRNAs and so on. All rAAV are premade, in stock, ready to ship, provided as 100ul aliquots at the titer of 1x10^12 GC (Genome Copies) /ml, measured by qPCR.

2. Select AAV products with desired Serotypes,

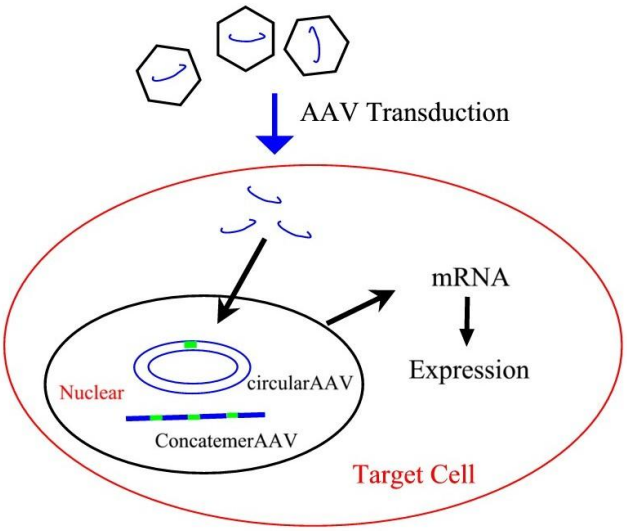
AAV serotypes refer to natural or engineered variants of AAV that differ in their capsid proteins, which dictate the viral tropism (preference for specific cell types or tissues). Gentarget Provides 11 Serotypes as: AAV1 to AAV10 and AAV-DJ.

Selecting the appropriate AAV serotype depends on the target tissue or cell type (refer to table below). If unsure, an effective approach is to test multiple AAV serotypes carrying a GFP reporter (CAT# *A1.12*: AV-GFP-kit) to determine the most efficient one for your system.

AAV Serotypes	Tissues or Cell Types Preferences (the most efficiently transduced tissues listed first)
AAV1	Skeletal Muscle, Heart ((vascular endothelial), cardiac muscle, CNS, Liver, Lung
AAV2	CNS (Brain), Retina, Kidney, Liver, Skeletal Muscle, Monocytes
AAV3	Liver, Kidney, Fibroblasts
AAV4	Retina, CNS (ependymal cells)
AAV5	Lung, CNS (brain and astrocytes), Retina, Ovary
AAV6	Lung (epithelial cells), Skeletal Muscle, Heart, Liver
AAV7	Skeletal Muscle, Liver, Heart
AAV8	Liver (hepatocytes), Heart, Skeletal Muscle, CNS, Kidney, pancreas
AAV9	CNS (can cross the blood-brain barrier), Heart (cardiac muscle), Skeletal Muscle, Liver, Lung
AAV10	CNS, Retinal ganglion cells, Heart, Skeletal Muscle,
AAV-DJ	CNS, Liver, Kidney, Fibroblasts (enhanced efficiency in the liver, CNS, and muscle)

3. AAV Transduction Protocol,

Premade AAV virus are provided as ready-to-use status and easy to use, no need other reagents or component. Simply add the rAAV to cell culture medium at the desired multiplicity of infection (MOI).



Notes:
(1) The optimal AAV dose depends on several factors, including target cell/tissue type, AAV serotype, and desired expression levels. It's often advisable to test multiple serotypes in your specific experimental system.
(2) A general starting point for in vitro experiments is MOI (GC/cell) = 10^5. For in vivo applications, typical doses range from 10^{11}–10^{13} GC per mouse, depending on the delivery method and target tissue.

AAV Transduction Protocol in 24-well plate:

Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight. So, at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells as: 2.5 x 10^5 cells/well (in 0.5ml medium) in 24-well plate. The cell number will be doubled after overnight culture.

Day 1:

- Thaw the rAAV stock at room temperature (or on ice), and add the appropriate amount of virus stock (**50ul/per well**, see calculation

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Fax: 1 (800) 380-4198
Email: orders@gentarget.com

below), to obtain the desired MOI (10^5). Return cells to 37°C, CO_2 incubator. Do nothing.

Note: Try to avoid freezing and thawing. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use;

(1) **MOI Selection**: The optimal MOI depends on your experimental goals and cell type. A commonly used for in vitro experiments is: MOI = 10^5.

(2) **Virus Volume Calculation**:

Desired MOI = 10^5
Cell Number = 5×10^5 (one well in 24-well plate)
Titer = 1×10^12 (GC/mL).

Virus Volume (mL/well) =10^5 × 5 × 10^5 / 10^12 = **50 ul (0.05 mL)**

(virus volume can be scaled up according to cell culture size)

Day 2 to 14: **Expression Analysis**:

Allow sufficient time for gene expression to occur. AAV Gene expression starts slower, generally within 2–14 days post-transduction, (up to 4 weeks, depend upon cell types). You may use the transduced cells for further applications.

4. Safety Precaution,

Premade rAAVs are replication incompetent. Use AAV in Bio-safety II cabinet. Wear glove all the time when handling AAV.

5. References,

(1) Current Gene Therapy, Volume 14, Issue 2, Apr 2014, p. 86 – 100; Basic Biology of Adeno-Associated Virus (AAV) Vectors Used in Gene Therapy
(2) Human Gene Therapy Methods 30, 206–213 (2019); A User's Guide to the Inverted Terminal Repeats of Adeno-Associated Virus.
(3) Human Gene Therapy, 2023, V34 (15, 16), Page 742-757; Improvement of Precision in Recombinant Adeno-Associated Virus Infectious Titer Assay with Droplet Digital PCR as an Endpoint Measurement.

6. Warranty,

This product is for research use only. It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall

7930 Arjons Drive, Suite B
San Diego, CA 92126, USA
Phone: 1 (858) 265-6446
Fax: 1 (800) 380-4198
Email: orders@gentarget.com

GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.