

pAAV-GFP Accessory Reagent

pAAV-GFP Control Vector

CATALOG #
AAA44597

PRODUCT SYNONYMS

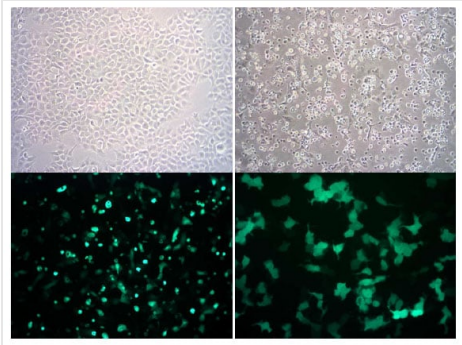
pAAV-GFP; pAAV-GFP Control Vector; pAAV-GFP accessory reagent

PRODUCT SPECIFICATIONS

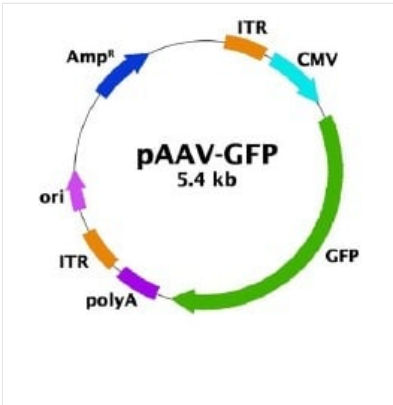
Concentration	10 ug at 0.25 ug/uL in TE (varies by lot)
RAAV Production	1. One day before transfection, plate sufficient 293 cells or 293AAV cells to achieve 70-80% confluence on the day of transfection. 2. Cotransfect cells with pAAV Expression vector, pAAV-RC2 and pHelper. Notes: 1) We recommend the ratio of vectors at 1:1:1 (pAAV Expression Vector:pAAV-RC2:pHelper). 2) 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: 1) 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. 2) 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 dryice/ethanol bath and a water bath of 37 degree 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 degree 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. I. 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 AAA Biotech's ViraBind AAV Purification Kit . II. Measure titer of your rAAV: 1) 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. AAA Biotech's QuickTiter AAV Quantitation Kit 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. 2) 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. III. 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. AAA Biotech's ViraDuctin AAV Transduction Kit is designed to increase transduction efficiencies by AAV on both dividing and non-dividing cells.
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.
Preparation and Storage	-20 degree C

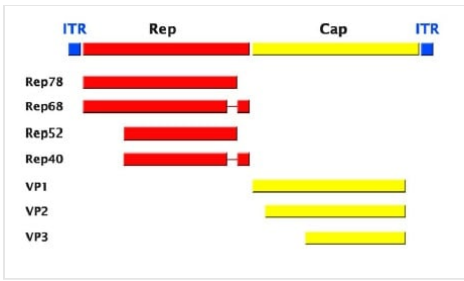
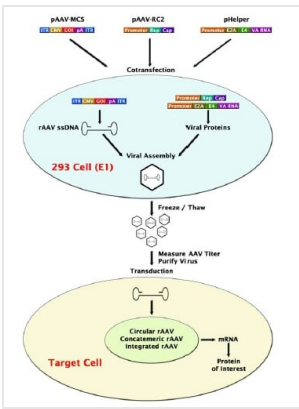
IMAGES



Application Data



Application Data



Application Data

Application Data

ADDITIONAL INFORMATION

Related Product Information for pAAV-GFP accessory reagent

- Use this control vector to co-transfect along with AAV packaging vectors to produce a recombinant AAV control.

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). AAA Biotech's 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-FreeSystem, 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.

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.

Product Categories/Family for pAAV-GFP accessory reagent

[Viral Expression](#); [Adeno-Associated Virus \(AAV\) Expression](#); [AAV Expression and Packaging](#); [AAV Control Plasmids](#)

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. Summerford, C., and Samulski, R. J. (1999) Nat. Med., 5, 587-588.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

AAA Biotech | Tel: +1 (619) 604-9440 | Fax: +1 (619) 604-9441 | contact@aaabiotech.com
Online datasheet: <https://stage.aaabiotech.com/accessory-reagent/paav-gfp/44597>