AAVanced Concentration Reagent

Cat# AAV100-SAM, AAV100A-1, AAV110A-1

User Manual

Store the reagent at room temperature

Version 8
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Contents

Product Description

Adeno-associated virus (AAV) is a single-strand DNA virus belonging to the *Parvoviridae* family. AAV can infect a wide range of cell types, including both dividing and quiescent cells. In humans, AAV infection is not associated with any known diseases, thus it has been widely adopted for delivery of recombinant DNA for *in vivo* applications. Recombinant adeno-associated virus (rAAV) vectors have been developed via removal of the AAV packaging signal from the virus genome. While the native virus integrates into a specific genomic locus in the host cell (*AAVS1*, located in chromosome 19q13.13), rAAV vectors persist in an extrachromosomal state with a very low frequency of random integration. Therefore, rAAV vectors have been broadly used in gene therapy and genome engineering as an alternative to other viral gene delivery methods.

Traditional rAAV production methods requires multiple steps, including cell lysis and $CsCl_2$ ultra-high-speed density gradient centrifugation, chromatography, or binding to affinity matrix columns. These are difficult to setup, time-consuming, and require specialized equipment for isolation of high-purity rAAV for *in vivo* experiments. Many studies have shown that several rAAV serotypes are efficiently released into the culture medium after co-transfection of the plasmids required to produce rAAV particles in human embryonic kidney 293 cells. To take full advantage of this property of rAAV, SBI has developed an innovative rAAV concentration solution called AAVanced™ Concentration Reagent – a simple, one step rAAV concentration reagent for the isolation of rAAV particles from media. AAVanced Concentration Reagent is based on a proven nanoparticle technology and is specifically optimized for the precipitation of most AAV serotypes from culture medium. The rAAV virus produced using AAVanced Concentration Reagent has been tested successfully for *in vitro* and *in vivo* applications for multiple serotypes of AAV with no observed cytotoxic effects, which provides direct evidence for the utility of this reagent for demanding applications using rAAV.

SBI's AAVanced Concentration Reagent significantly reduces the complexity and time required for rAAV virus production, allowing researchers to focus on their research experiments rather than virus production.

List of Components

Catalog Number	Description	Amount	Storage Condition
AAV100-SAM	AAVanced Concentration Reagent	5 mL	Room temperature
AAV100A-1	AAVanced Concentration Reagent	100 mL	Room temperature
AAV110A-1	AAVanced Concentration Reagent	250 mL	Room temperature

Storage

The kits are shipped at room temperature or on blue ice and should be stored at RT upon receipt. Properly stored kits are stable for 1 year from the date received.

Equipment to be supplied by user

1. HEK293TN cells (Cat #LV900A-1, System Biosciences). Other low passage HEK293T or FT cells can also be used if available.
2. AAV shuttle and packaging vectors (including plasmids encoding for Rep/Cap protein and helper factors (e.g. Adenovirus E2A, VA, and E4 proteins)
3. 150mm^2 tissue-culture plates
4. PureFection transfection reagent (Cat #LV750A-1, System Biosciences). Other reagents such as Lipofectamine 2000 may also be suitable.

Protocol:

AAV Concentration Protocol

AAVanced™ Concentration Reagent is provided in a 5x solution.

1. Seed 7 x 10^6 HEK293TN cells in 150mm^2 plate(s) with 20ml of complete growth medium (i.e. DMEM, 10% FBS) without antibiotics to reach 70-80% confluency within 24hrs.
2. Transfect cells with manufacturer recommended amounts of AAV shuttle, Rep/Cap, and AAV helper plasmids for 150mm^2 plates using PureFection™ (Cat # LV750A-1, please visit SBI website for detailed protocol)
3. Next day after transfection, change the media to complete growth media including antibiotics.
4. 72 hours after initial transfection, transfer supernatant with free rAAV virus to a sterile 50ml conical vessel. If using multiple plates, the supernatants can be combined together before addition of AAVancedTM Concentration Reagent
5. Centrifuge at 3000 × g for 15 minutes to remove cells and cell debris.
6. Add 1 volume of cold AAVancedTM Concentration Reagent (4ºC) to every 4 volumes of AAV particles-containing supernatant. Mix thoroughly with pipetting (For example, add 2 ml AAVancedTM Concentration Reagent for every 8 ml of supernatant)

7. Refrigerate overnight (or at least 12 hours). AAV viral particles-containing supernatants mixed with AAVanced™ Concentration Reagent are stable for up to 4-5 days at 4°C.

8. Centrifuge supernatant/ precipitation mixture at 1500 × g (or 3000 rpm) for 30 minutes at 4ºC. After centrifugation, the AAV viral particles may appear as a beige or white pellet at the bottom of the vessel.

9. Remove all of the supernatant (or as much as possible) and spin the tube containing AAV pellet again 1500 × g (or 3000 rpm) for 3 minutes at 4ºC. Completely remove all traces of fluid by aspiration.

10. Resuspend viral pellets in 1/1000 (for use in vivo) to 1/100 (for use in vitro) of original volume using cold, sterile Phosphate Buffered Saline (PBS) or DMEM containing 25mM HEPES buffer at 4ºC. Titer the concentrated AAV with a method of your choice.

11. Aliquot into cryogenic vials and store at -80°C until ready for use.

Precipitation of AAV virus particles from large volumes can be achieved by using the Corning 250 mL polypropylene centrifuge tube (Cat. # 430776), following manufacturer's instructions.

General rAAV Transduction Protocol

A general protocol for transduction of rAAV particles is shown below. This protocol can be scaled upwards with respect to vessel size.

1. Day 1: Plate 10,000 cells per well in a 24 well plate (about 30% confluence) in culture medium.

2. Day 2: Add virus to each well at different volumes based on the experimental needs (typical amount of virus added will range from 1 – 10 ul for a single well in 24-well plate)

3. Day 3: Change to complete growth medium

4. Day 6-7: Look at the cells for reporter expression if the rAAV construct has a reporter (e.g. GFP) and/or begin appropriate antibiotic selection to establish stable cell line.

Example Data and Applications

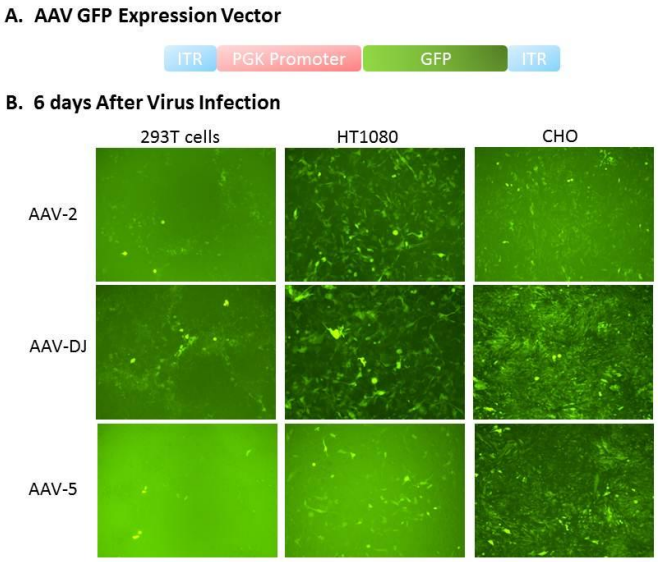

Fig. 1. Schematic diagram of the rAAV construct used *in vitro* and *in vivo* experiments is shown in Figure 1A. Three AAV serotypes (AAV-2, AAV-DJ and AAV-5) were precipitated using AAVanced™ Concentration Reagent. Three cell lines (HEK293T, CHO and HT1080) were used to validate the transduction efficiency of virus isolated with AAVanced™ Concentration Reagent *in vitro*. 1 ul of precipitated virus was used to infect cells, and images are taken 6 days after virus infection (Figure 1B).

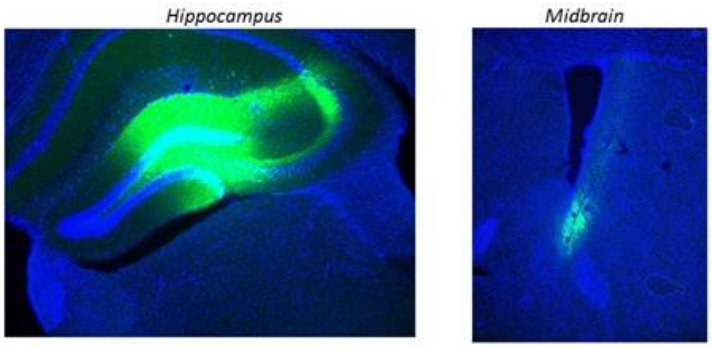

Fig. 2. Representative images of hippocampal and midbrain sections from 6 week old C57 mice injected with AAV virus (ITR-PGK-GFP-ITR) concentrated using AAVanced™ Concentration Reagent. 1.5 ul of concentrated AAV virus (AAV-2 serotype) was delivered to hippocampal and midbrain regions by stereotaxic injection. Three weeks after virus injection animals are perfused by paraformaldehyde, fixed, and brains are sectioned to 40 micron slices before visualization.

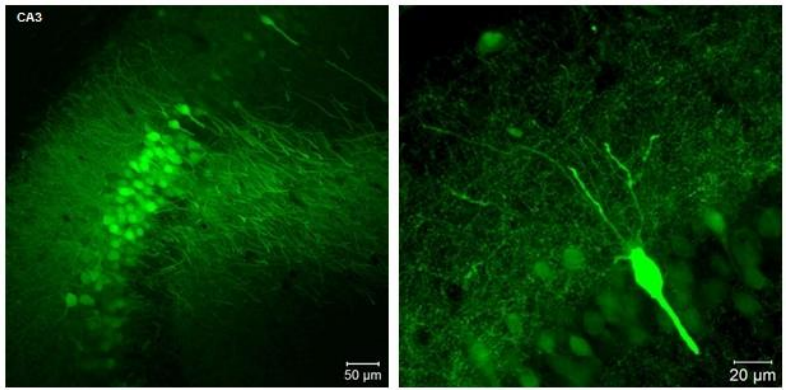

Fig. 3. Representative images of mouse neurons in cortical sections of 2 month old C57 mice injected with AAV virus (ITR-PGK-GFP-ITR) concentrated using AAVanced™ Concentration Reagent. 1.5 ul of concentrated AAV virus (AAV-5 serotype) is delivered to cortical region by stereotaxic injection. Two weeks after virus injection animals are perfused by paraformaldehyde, fixed and brains are sectioned to 70 micron slices before visualization.

References

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3. Vandenberghe LH et al. Efficient serotype-dependent release of functional vector into the culture medium during adeno-associated virus manufacturing. Hum Gene Ther. 2010 Oct;21(10):1251-7.

4. Lock M et al. Rapid, simple, and versatile manufacturing of recombinant adeno-associated viral vectors at scale. Hum Gene Ther. 2010 Oct;21(10):1259-71

5. Doria M et al. AAV2/8 vectors purified from culture medium with a simple and rapid protocol transduce murine liver, muscle, and retina efficiently. Hum Gene Ther Methods. 2013 Dec;24(6):392-8.

Technical Support

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