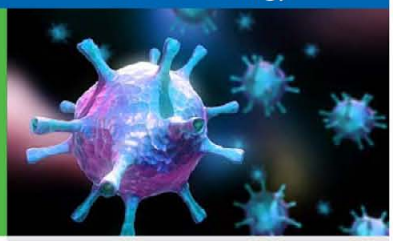
SBI System Biosciences
Harnessing innovation to drive discoveries

AAV Technology

AAVanced™ Concentration Reagent

One-step rAAV particle isolation from packaging cell media



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.

Highlights

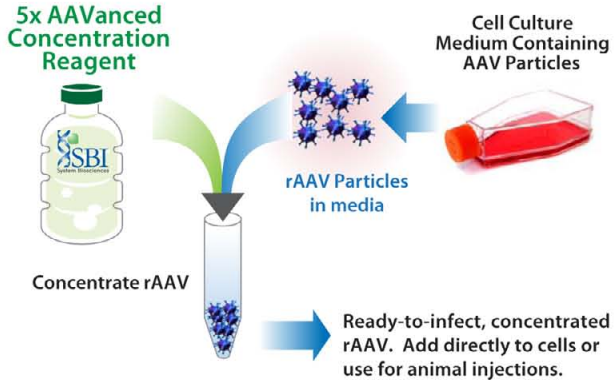
- Easy to use, single reagent
- Isolate rAAV directly from media
- No ultracentrifugation or cell lysis required
- Saves time and cost-effective
- Works with all rAAV serotypes
- Compatible with standard rAAV packaging plasmid mixes
- Non-toxic to transduced cells

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. As many studies have shown that different rAAV serotypes are efficiently secreted into the culture medium of transfected 293T cells during rAAV packaging, SBI has developed a novel reagent to facilitate fast, high-quality rAAV isolation.

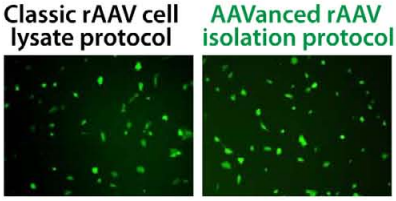
Easiest Protocol to Isolate rAAV Particles

SBI has developed an innovative 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.

LEARN MORE:
www.systembio.com/aav



AAVanced rAAV Particles are Robustly Infective

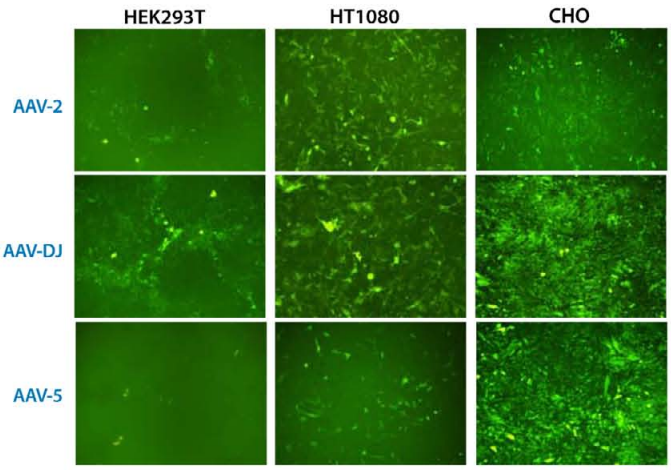


rAAV shuttle vector used in comparison tests

Recombinant rAAV was packaged with a PGK-GFP expression shuttle vector. The rAAV particles were isolated either by the traditional cell lysis freeze-thaw with centifugation method or with the AAVanced Concentration Reagent from the packaging cell media. Equal amounts of rAAV were then added to HEK293 cells to test for infectivity based on GFP expression. Representative images of GFP expressing cells 10 days after infection.

AAVanced rAAV Isolation Works with Multiple Serotypes *In Vitro*

Recombinant AAV was packaged in parallel with three different serotypes: AAV-2, AAV-DJ and AAV-5. The exact same shuttle rAAV vector with a PGK promoter expressing GFP was used for all tests. The media was collected after 48 hours and the rAAV particles in the media were isolated using the AAVanced Concentration Reagent. Each rAAV particle pellet was resuspended in 100µl sterile PBS and then 1µl of the rAAV suspensions were added to three different cell lines (HEK293T, HT1080 and CHO) to validate transduction efficiencies. The infected cells were imaged for GFP expression 6 days after addition of the isolated rAAV particles.

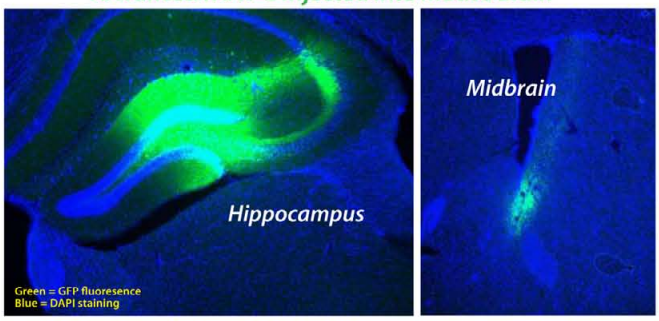


AAVanced rAAV Particles Work *In Vivo*

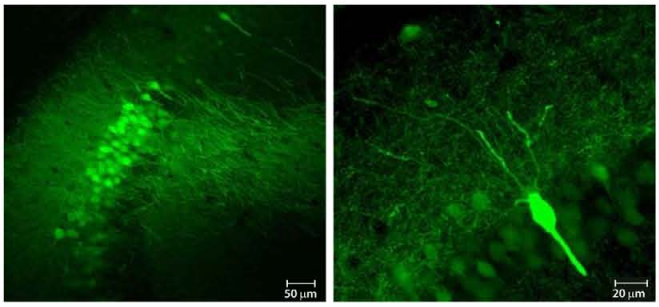
Shown in the upper right panels are representative images of hippocampal and midbrain sections from 6 week old C57 mice injected with AAV virus (ITR-PGK-GFP-ITR) concentrated using AAVanced Concentration Reagent. Approximately 1.5µl of concentrated AAV virus (AAV-2 serotype) was delivered to hippocampal and midbrain regions by stereotaxic injection. Three weeks after virus injection, the animals were perfused with paraformaldehyde, fixed, and brains specimen were sectioned to 40 micron slices before visualization for GFP fluorescence.

Shown in the lower right panels are representative images of mouse neurons in cortical sections of 2 month old C57 mice injected with AAV-5 virus (ITR-PGK-GFP-ITR) concentrated using AAVanced Concentration Reagent. Approximately 1.5µl of concentrated rAAV-5 virus was delivered to cortical regions by stereotaxic injection. Two weeks after virus injection, animals were perfused by paraformaldehyde, fixed and brains were sectioned to 70 micron slices before visualization for GFP fluorescence.

AAVanced rAAV-2 Injected into Mouse Brain



Mouse Cortical Neurons Infected with AAVanced rAAV-5



Images courtesy of Dr. Woo-Ping Ge's lab at UT Southwestern.

We Also Offer Custom AAV Cloning and Production Services

System Biosciences offers a wide-range of custom services to support your research, allowing you to spend less time making tools, and more time making discoveries. To learn more, visit our website at www.systembio.com .

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