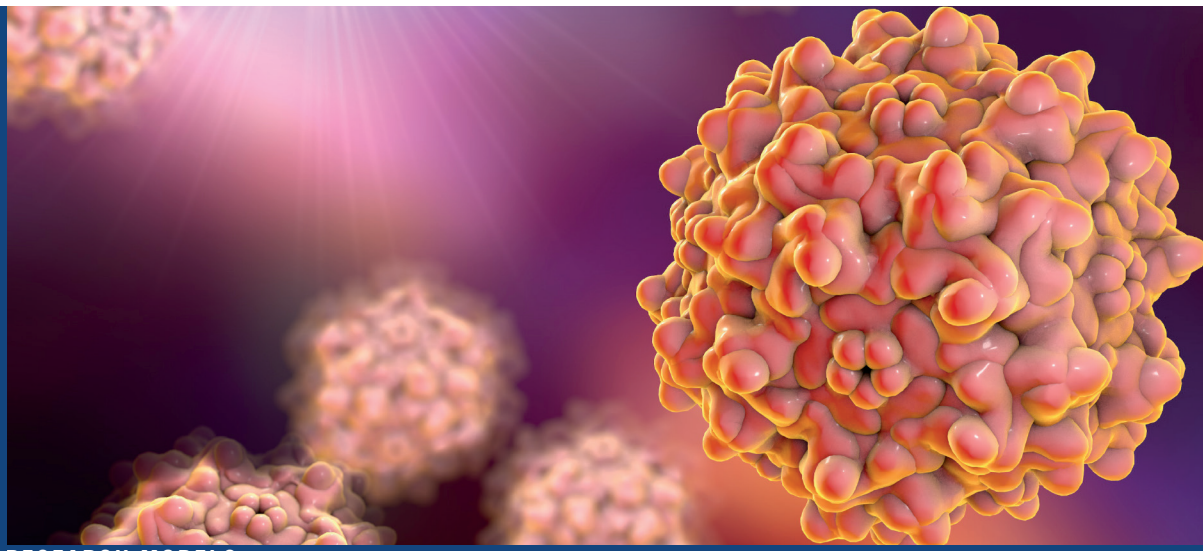
charles river

Summary
Tips and guidance about AAV transduction and injection protocols.



RESEARCH MODELS

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Adeno-associated Virus (AAV) Protocols

Before you get started

- **Do your research -** The following protocols are intended to be general guidelines and are not optimized for your specific cell line or animal model. We recommend that you do a literature search to find a protocol that closely aligns with your experimental conditions for optimal results.
- **Aliquot the virus -** To avoid repeated freeze-thaw cycles that can decrease the viral titer, aliquot the AAV stock upon arrival and keep the aliquots at -80°C for long-term storage. Once an aliquot is thawed, it may be stored at 4°C for several weeks without significant loss of biological activity.

Transduction (*in vitro*)

The optimal concentration of AAV is highly variable depending on the serotype, cell type, and experimental conditions. A range of 1,000-50,000 multiplicity of infection (MOI) is typically used for readily transducible cell lines; however, a MOI of up to 2,000,000 may be required for some cell lines. To determine the optimal concentration of AAV to use, we recommend you conduct a pilot experiment using a reporter control AAV that has the desired serotype and promoter (e.g., AAV2-CMV-GFP).

After you decide on the MOI to use in your experiments, dilute the viral stock in medium to achieve the desired MOI. Remove the culture medium and add the AAV-containing medium to the cells using the minimum amount necessary to cover the well/dish. After 6-12 hours, you can exchange or add media to the well/dish. Look for expression at 24 h, 48 h, 72 h, 96 h, or at your desired time points.

Calculating the Volume of Virus Needed

AAV GC needed = Desired MOI x Number of cells

E.g., If your desired MOI is 10,000 and you want to transduce 1,000,000 cells, you need 10^{10} GC. If the titer is $1x10^{13}$ GC/mL, add 1 μL of the AAV stock to the medium.

Plate/Dish Size	Media Volume
24 well plate	0.25-0.5 mL
12 well plate	0.5-1 mL
6 well plate	1-2 mL
60 mm dish	3-4 mL
10 cm dish	8-12 mL

Injection (*in vivo*)

To determine the optimal amount of AAV to use for mice or other small animal injections, we recommend first testing three doses at 10^{11}, 10^{12}, and 10^{13} GC/kg. Assuming your mice are 20 g, the doses would be $2x10^{9}$, $2x10^{10}$, and $2x10^{11}$ GC/mouse. If you are doing a non-localized injection, you may also want to include an even higher dose, such as $4x10^{11}$ GC/mouse. Please note that these are general guidelines for mice and may not apply to larger animal models (e.g., pigs, non-human primates).

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