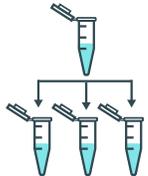


Before you get started



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 1×10^{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 2×10^9 , 2×10^{10} , and 2×10^{11} GC/mouse. If you are doing a non-localized injection, you may also want to include an even higher dose, such as 4×10^{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).

www.vigenebio.com



Local 301-251-6638
Toll Free 1-800-485-5808



Orders orders@vigenebio.com
Support custsupport@vigenebio.com



@VigeneBio



Vigene Biosciences