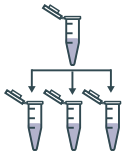


## Before you get started



### 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 viral stock upon arrival and keep the aliquots at  $-80^{\circ}\text{C}$  for long-term storage.



### Follow biosafety procedures

Adenoviruses are classified as BSL-2 organisms, so be sure to follow the recommended NIH guidelines. This includes the required training and use of a Class II biological safety cabinet.

## Transduction (in vitro)

If this is your first time transducing your adenoviral construct into the cell line, we recommend conducting a pilot experiment using a reporter control (e.g., Ad-GFP) to determine the MOI that gives the highest transduction efficiency with minimal cell death. We recommend using MOIs of 0 (uninfected control), 10, 30, 100, or even higher (up to 100,000) for hard-to-transduce cell lines.

After you decide on the MOI to use in your experiments, dilute the viral stock in medium (do not vortex) to achieve the desired MOI. Remove the culture medium and add the adenovirus-containing medium to the cells using the minimum amount necessary to cover the well/dish. Incubate cells at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$  overnight. The next day, exchange virus-containing medium for fresh complete medium. Look for expression at 24 h, 48 h, 72 h, or 96 h post-transduction or at your desired time points.



### Calculating the Volume of Virus Needed

Adenovirus VP needed = Desired MOI x Number of cells

E.g., If your desired MOI is 10 and you want to transduce 1,000,000 cells, you will need  $10^7$  VP. If the titer is  $1 \times 10^{10}$  VP/mL, add 1  $\mu\text{L}$  of the viral 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 amount of adenovirus to use for mice or other small animal injections, we recommend first testing three or more doses. Assuming you are using average-sized adult mice, doses of  $10^9$ ,  $10^{10}$ , and  $10^{11}$  VP can be used as starting points to find the optimal dose. Please note that these are general guidelines for mice and may not apply to larger animal models, such as pigs or non-human primates.

[www.vigenebio.com](http://www.vigenebio.com)



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



Orders [orders@vigenebio.com](mailto:orders@vigenebio.com)  
Support [custsupport@vigenebio.com](mailto:custsupport@vigenebio.com)



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