

## Adeno-associated Virus (AAV) Infection Protocol

**Storage Recommendations:** Adeno-Associated Virus (AAV) stocks are supplied in liquid form. Keep the stocks at  $-80^{\circ}\text{C}$  for long term storage. Aliquots of the Adeno-Associated Virus (AAV) stocks are recommended to avoid titer reduction from multiple freeze-thaw cycles.

**Prior to transduction, prepare the virus stock with growth medium for the desired cell line by following the steps below:**

1. Thaw the Adeno-Associated Virus (AAV) viral stock at room temperature or on ice.
2. Calculate the appropriate volume of virus needed to be diluted into the media in order to achieve the desired MOI (Multiplicity of infection) of virus.

**MOI = AAV GC particles needed / Number of cells to be infected**

To infect 1 million cells with desired MOI of 10,000, the amount of virus needed =  $10,000 \times 1,000,000$  which =  $10^{10}$  GC. If the original stock is  $10^{13}$  GC/ml, then you will need 1ul of the original stock for dilution.

**Note:** Adeno-Associated Virus (AAV) MOI ranges from 10,000 to 500,000 depending on serotype and cell type. The appropriate amount of viruses needed for the infection is crucial to the experimental result. Thus, it is strongly recommended to infect your target cells with a reporter AAV control virus relevant to desired serotype, in your preliminary study to determine the optimal MOI.

3. Dilute AAV into corresponding media as calculated in previous step.

**Once the virus is prepared, infect the cells with virus containing media following the steps below:**

4. Remove the original cell culture medium.
5. Add AAV-containing medium to cell culture with the recommended volume shown in the table:

Plate Size	Volume of Virus Containing Media
24-well plate	0.5 ml
12-well plate	1 ml
6-well plate	2 ml
60 mm-plate	5 ml
10 cm-plate	10 ml

6. Incubate cells with the virus containing medium at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$  for 6-12 hours or longer depending to your experimental design.

7. As a general guideline only, in 1-2 weeks you may use the transduced product for further applications.

**Note:**

- (1) AAV stock can be added directly to cells in culture medium (in the presence or absence of serum).
- (2) It is not necessary to remove viruses, change or add media following infection. Although viruses can be removed after 6-12 hours post infections.
- (3) It can take 3-7 days after the AAV infection to detect gene over expression