# Adeno-Associated Virus (AAV) Viral Infection Guideline

**Important notes**:
Adeno-Associated Virus (AAV) stocks are supplied in liquid form. Keep the stocks at -80°C for long term storage. Aliquots of the Adeno-Associated Virus (AAV) stocks are recommended to avoid titer reduction from multiple freeze-thaw cycles.

## Important Guidelines

***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 (Multilicity of infection) of virus.

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

eg. To infect 1 million cells with desired MOI of 10,000, the amount of virus needed = 10,000X1,000,000 which = $10^{10}$ GC.

Note: Adeno-Associated Virus (AAV) MOI ranges from 10,000 to 500,000 depending on serotype and cell type. It is strongly recommended to infect your target cells with a GFP reporter AAV control virus relevant to desired serotype (eg. Scramble AAV siRNA Control Virus (Serotype 2)) in your preliminary study to determine the optimal MOI.

3. Dilute AAV into corresponding media as calculated in previous step. If transduction signal is low, one can also add in enhancers such as AAViralEntry™ Transduction Enhancer (**abm**, Cat# G516).

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

| Plate Size | Volume of Virus containing media |
|---|---|
| 24-well plate | 0.2-0.3 ml |
| 12-well plate | 0.5-0.8 ml |
| 6-well plate | 1-1.5 ml/ well |
| 60 mm-plate | 3-4 ml/ plate |
| 10 cm-plate | 8-12 ml/ plate |

4. Remove the original cell culture medium.

5. Add AAV-containing medium to cell culture with the recommended volume shown in the table:
6. Incubate cells with the virus containing medium at 37°C with 5% $CO_2$ according to your experimental design.

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

Reference:

Ellis et al. A survey of ex vivo/in vitro transduction efficiency of mammalian primary cells and cell lines with Nine natural adeno-associated virus (AAV1-9) and one engineered adeno-associated virus serotype. Virology Journal. 2013. 10:74

Hilda et al. High-efficiency Transduction of the Mouse Retina by Tyrosine-mutant AAV Serotype Vectors. The American Society of Gene Therapy. March 2009. 17:3. Pg 463-471.

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