

User Manual for Ready-to-use AAV Products

CONTENTS AND STORAGE

AAV stocks are supplied in liquid form at indicated titer. The storage solution PBS / 5% glycerol. Store at -80°C. If desired, aliquot viral stock upon arrival, and store those aliquots at -80°C freezer immediately. **DO NOT FREEZE AND THAW REPEATEDLY.**

IMPORTANT GUIDELINES

Follow these guidelines when performing infections:

1. Prepare virus-containing media:

Thaw viral stock at either room temperature or on ice.

Add desired amount of virus to growth media to achieve the desired MOI.

AAV GC particles to be used = MOI (multiplicity of infection) * # of cells to be infected

e.g. If you intend to infect 1 million cells using MOI of 1,000, you need $1,000 \times 1,000,000 = 10^9$ GC for the infection. If the original stock is 10^{13} GC/ml, then you will need 0.1 ul of the original stock for the dilution.

2. Infecting cells with virus:

Remove the original cell culture media, and add the above AAV-containing media to cell culture. Below is a general guideline for the amount of media used:

| | |
|----------------|---------------|
| 24-well plate: | 0.2-0.3 ml |
| 12-well plate: | 0.5-0.8 ml |
| 6-well plate: | 1-1.5 ml/well |
| 60mm-plate: | 3-4 ml/plate |
| 10cm-plate: | 8-12 ml/plate |

Incubate cells with the virus-containing media for 6-12 hours, or as long as you wish.

(Optional), you could remove virus-containing media and replace it with fresh, desired media.

The appropriate amount of viruses used for infecting cells is critical for the outcome of your experiments. The goal is to get 100% of infection without causing any undesired effects. The optimal concentration differs dramatically between cell types for different serotype of AAV. A range of 2,000-50,000 MOI is used for most cell lines for AAV2, but up to 500,000 MOI may be used for some cells.

To determine this optimal concentration of virus for your study, you could conduct pilot testing in your cell line by using reporter AAV like AAV-GFP.

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 medium 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 the gene over-expression

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