



AAVPrime™ AAV Serotype Testing Kit

Cat. No. AAV320

AAVPrime™ AAV Serotype Testing Kit Plus

Cat. No. AAV321

Protocol: Transduction of Target Cells with AAV

I. Introduction

GeneCopoeia's AAVPrime™ Adeno-associated virus (AAV) Serotype Testing Kit (Cat. No. AAV320) contains 9 premade GFP-expressing AAV in serotypes 1, 2, 3, 6, 7, 8, 9, DJ and DJ/8. AAVPrime™ Adeno-associated virus (AAV) Serotype Testing Kit Plus (Cat. No. AAV321) contains 12 premade GFP-expressing AAV in serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, DJ and DJ/8. All AAV particles are offered at 5×10^{12} GC/ml, in 25 μ l stocks, except that serotype 4 and 10 are offered at 5×10^{11} - 10^{12} GC/ml. These kits are ideal for testing the infection efficiency of AAV serotypes on different cell types.

II. Experimental Procedure

The procedure below is based on the transduction of HT1080 cells with the AAV Serotype Testing Kit Plus in 24 well plates. Other cells can also be used, but efficiencies may vary up to several orders of magnitude.

Day 1: Plate cells to be transduced

1. Plate $\sim 1.3 \times 10^5$ cells per well in a 24-well plate 24 hours prior to viral infection. Use 1 ml of culture medium supplemented with 10% heat-inactivated fetal bovine serum for each well. Incubate the cells at 37 °C with 5% CO₂ overnight until 80% confluent.

Day 2: Transduce target cells

2. (Optional) Treat cells with 0.8 μ M camptothecin in the medium. Mix well by swirling, then

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return the plates to the 37 °C incubator for 4 hours. This step is optional but can increase the transduction efficiency for many cell lines.

3. Calculate the volume of viral stock to be added to the cells according to the desired multiplicity of infection (MOI).

$$\text{MOI} = \text{AAV GC particles needed} / \text{Number of cells to be infected}$$

For an MOI = 1, the volume (in μl) of AAV particles needed = ((total number of cells per well)/(number of genome copies (GC)/ml)) x 1,000.

The MOI used is critical to achieve 100% infection of the target cells without causing major side effects. A range of MOI from 1,000 to 10,000 is suggested for most cell lines, however, up to 500,000 MOI may be needed for some cell line with some specific serotype AAV particles.

To determine the MOI needed when transducing a cell line for the first time, we recommend first to transduce the target cells with the same serotype eGFP reporter AAV particles at a series of MOIs, such as MOIs of 100, 1,000, and 10,000, or even higher for some cell lines that are difficult to be transduced.

Example: 3×10^5 HT1080 cells were prepared for transduction by AAV particles (1×10^{11} GC/ml) one day after plating. For an MOI = 1,000, 3 μl AAV particles are needed. For an MOI = 10,000, 30 μl AAV particles are needed.

4. Thaw the AAV particles at room temperature. Dilute the viral stock to the needed MOI in 200 μl culture medium supplemented with 2% (V/V) heat-inactivated FBS.
5. Aspirate the medium from the camptothecin-treated (optional) cells. Add the 200 μl of diluted virus to the cells.
6. Incubate 2 hours at 37 °C in a CO₂ incubator, swirling the plate every 30 minutes.
7. Add 200 μl pre-warmed culture medium with 18% (V/V) heat-inactivated FBS, and incubate for 40-48 hours.

Day 4: Replace medium

8. Remove the medium containing AAV particles from the wells and replace with 1 ml fresh pre-warmed culture medium with 10% (V/V) heat-inactivated FBS.

Day 5 to 14:

9. Harvest the cells for further investigation.

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