AAVPrime™ Adeno-associated virus (AAV) Particles

Protocol: Transduction of Target Cells with AAV

I. Introduction

GeneCopoeia's AAVPrime™ Adeno-associated virus (AAV) products are the ideal tools for inserting genes into a broad range of cell types with high efficiency and enhanced safety. GeneCopoeia's optimized helper-free human AAV system allows viral packaging without potentially pathogenic helper adenovirus.

II. Experimental Procedure

The procedure below is based on the transduction of HT1080 cells (such as GCI-HT1080 cells) with AAV2 serotype in 24 well plates. Other cells and serotypes can also be used, but efficiencies may vary up to several orders of magnitude.

For serotypes other than AAV-2, it is recommended to select a cell type that can be well transduced by that specific AAV serotype.

Day 1: Plate cells to be transduced

1. Plate ~ 1.3 x 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 ºC with 5% CO_2 overnight until 80% confluence.

Day 2: Transduce target cells

2. Treat cells with 0.8 µM camptothecin in the medium. Mix well by swirling, then return the plates to the 37 ºC 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).

   MOI = AAV GC particles needed / 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 of 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 x 10^5 HT1080 cells were prepared for transduction by AAV particles (1 x 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 cells. Add the 200 µl of diluted virus to the cells.

6. Incubate 2 hours at 37 ºC in a CO_2 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 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.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at (301)-762-0888.

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