

Adeno-Associated Virus (AAV) Packaging Protocol

The following protocol allows for the production of recombinant Adeno-Associated Virus (AAV) viral particles up to a 10^9 GC/mL titer. We recommend including a negative control (without DNA or transfection reagents) in your experiments to help evaluate your results.

Before starting the Adeno-Associated Virus (AAV) packaging protocol, please ensure that you have an adequate volume of AAV plasmid DNA containing the gene of interest (10 μ g plasmid per 15 cm dish) and AAV Packaging Mix (30 μ g plasmid per 15 cm dish).

Protocol

This protocol serves as a general guideline to produce 10^9 GC/mL AAV using one 15cm dish. If needed, same ratio can be applied to scale up accordingly.

DAY 1:

1. In the afternoon, seed $\sim 1.2 \times 10^7$ of HEK293 cells in one 15 cm dish.

DAY 2: (Carry out steps 2 - 6 in the morning on the day of transfection)

2. Check to make sure the cells are 70-80% confluent.

3. **For each 15 cm dish** prepare the transfection complex as follows:

a) Solution A: Dilute 10 μ g of gene of interest; and 30 μ g of abm's AAV Packaging Mix (Cat. No. AAV1001, AAV1002, AAV1003, AAV1004, AAV1005, AAV1006) in 3 mL serum-free, antibiotic-free medium

Solution B: Dilute 227 μ L of DNAfectin™ Transfection Reagent (G2100) in 3 mL serum-free, antibiotic-free medium.

b) Incubate both solutions at room temperature for 5 minutes.

c) Combine solutions A and B together, mix well, and incubate at room temperature for 20 minutes. This will create the transfection complex.

4. Add 12 mL serum-free antibiotic-free medium to the transfection complex.

5. Remove medium from the cells in the 15 cm dish.

6. Add the complete transfection complex from step 4 to the cells and incubate at 37°C for 5-8 hours. Avoid dislodging the cells by gently adding the mixture against the sidewall of the dish.

7. Add 1.5 mL of FBS to the 15 cm dish and incubate at 37°C overnight.



DAY 3 / DAY 4:

8. Monitor the cells for any cytopathic effects. It is normal to observe the CPE around day 3/ day 4.

9. Incubate the cells at 37°C until day 5.

DAY 5 (Harvest):

10. Harvest the cells by using a cell scraper or trypsin-EDTA. Pellet the cells by centrifugation at 3000 rpm for 15 minutes at 4°C.

11. Discard the supernatant and wash the cell pellets 2 times with 250 µl of 1X sterile PBS. Pellet the cells by centrifugation at 3000 rpm for 15 minutes at 4°C.

12. Freeze-thaw cell pellets 3 times at -80°C and 37°C to release the virus into the supernatant. Vortex the cell pellets in between the freeze-thaw cycle.

13. Centrifuge the cell pellets at 10,000 rpm for 20 minutes.

14. Filter the cleared supernatant with a low-protein binding 0.45 µm sterile filter.

15. The filtered supernatant will be ready for further purification or other downstream application. Alternatively, it can be stored at -80°C as viral stock. Aliquotted volumes are preferred for long term storage to reduce the loss of viral titer through multiple freeze-thaw cycles.



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