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 expression DNA for gene of interest (10 µg plasmid per 15 cm dish); AAV helper plasmid (20 µg plasmid per 15 cm dish); and choice of desired serotype plasmid (10 µg 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 ~1.2 x 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: Dilute10 µg of gene of interest; 20 µg AAV helper plasmid ; and10 µg choice of desired serotype plasmid in 3 mL serum-free, antibiotic-free medium.

Solution B: Dilute 227 µL of DNAfectin™ Plus Transfection Reagent (G2500) 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 side wall of the dish.

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

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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 cycles.

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.