AAV Storage & Infection Protocol

Adeno-associated Virus (AAV) Protocol for In Vitro Application

- Storage Instructions and Infection Protocol

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This product is for laboratory research ONLY and not for diagnostic use

Storage:

Recombinant adeno-associated viruses though replication deficient, they remain infectious and should be handled as such.

1. Upon receiving, the virus should be stored at –80 °C.
2. Vectors are provided in 30 µl to 100 µl aliquots. After the first freeze/thaw cycle, dispense the vector in 10 µl aliquots or larger, depending on the amount to be used in your experiments.
3. Aliquots must be stored at –80 °C.
4. The vector titer starts to drop after the third freeze/thaw cycle.

In-vitro infection Protocol:

Required Materials:

- Recombinant AAV (rAAV) vector stock
- Target cells (HEK293, HeLa, SH-SY5Y, primary cells)
- Appropriate culture medium
- PBS (sterile)
- Trypsin or cell dissociation reagent
- Tissue culture plates

Cell Preparation:

- Seed cells one day prior to infection
- Aim for 60–80% confluency at infection time

Typical seeding densities:

- 6-well: $2–4 \times 10^5$ cells
- 12-well: $1–2 \times 10^5$ cells
- 24-well: $\sim 5 \times 10^4$ cells

MOI Selection:

Typical MOI ranges:

- Easy cell lines: $10^3–10^4$ VG/cell
- Moderate cell lines: $10^4–10^5$ VG/cell
- Primary cells: $10^5–10^6$ VG/cell

Virus Preparation:

- Virus volume calculation:
- Volume = (MOI × number of cells) / viral titer
- Dilute rAAV stock into culture medium to the desired infection volume.

Infection Procedure:

- Remove existing culture medium
- Add virus-containing medium
- Gently swirl plate to distribute virus
- Incubate at 37 °C, 5% CO_2

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Post-Infection Culture:
- Optional: Replace medium after 6 ~ 12 hours
- Early expression: ~24 hours
- Robust expression: 48 ~ 72 hours
- Peak expression: 4 ~ 7 days

Optional Enhancements:
- Spinfection: 1000 × g for 30–60 min
- Reduced infection volume
- Plate coating (poly-L-lysine)
- Optimize AAV serotype
- Co-infection with wild type adenovirus:

  Together with diluted AAV, co-infect with human adenovirus 5 wild type (SKU # SL101740) at MOI of 10~300. Co-infection with wild type adenovirus will significantly (5 ~ 100 folds) boost transduction efficiency and gene expression level.

Example Calculation:
Cells: 1×10^5
MOI: 5×10^4 VG/cell
Virus titer: 1×10^{13} VG/mL
Required virus volume ≈ 0.5 µL

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 4 ~ 7 days after the AAV infection to detect the gene over-expression
(4) Co-infection with wild type adenovirus will significantly boost AAV's transduction efficiency and transgene expression level.

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