User Manual of rAAV

# - Storage Instructions and Infection Protocol -

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## Contents and Storage:

AAV stocks are supplied in liquid form at indicated titer. The storage solution PBS / 5% glycerol. Store at -80 $^0$C. If desired, aliquot viral stock upon arrival, and store those aliquots at -80 $^0$C freezer immediately. ***DO NOT FREEZE AND THAW REPEATEDLY***.

## *In-vitro* Infection Protocol:

**1. Prepare virus-containing media:**

Thaw viral stock at either room temperature or on ice.
Add desired amount of virus to growth media to achieve the desired MOI.

AAV GC particles to be used = MOI (multiplicity of infection) x number of cells to be infected

e.g. If you intend to infect 1 million cells using MOI of 10,000 , you need 10,000 x 1,000,000 = $10^{10}$ GC for the infection. If the original stock is $10^{13}$ GC/ml , then you will need 1.0 ul of the original stock for the dilution.

**2. Infecting cells with AAV:**

Remove the original cell culture media, and add the above AAV-containing media to cell culture. Below is a general guideline for the amount of media used:

| | |
|---|---|
| 24-well plate: | 0.2-0.3 ml |
| 12-well plate: | 0.5-0.8 ml |
| 6-well plate: | 1-1.5 ml/well |
| 60mm-plate: | 3-4 ml/plate |
| 10cm-plate: | 8-12 ml/plate |

Incubate cells with the virus-containing media for 6-12 hours, or as long as you wish.
(Optional), you could remove virus-containing media and replace it with fresh, desired media.

The appropriate amount of viruses used for infecting cells is critical for the outcome of your experiments. The goal is to get 100% of infection without causing any undesired effects. The optimal concentration differs dramatically between cell types for different serotype of AAV. A range of 2,000-10,000 MOI is used for most cell lines, but up to 500,000 MOI may be used for some cells.

To determine this optimal concentration of virus for your study, you could conduct pilot testing in your cell line by using reporter AAV like AAV-GFP.

**3. Co-infection with Adenovirus**

Together with diluted AAV, co-infect with wild-type adenovirus type 5 (Ad-Null, Cat # SL100705) at MOI of 1~100. Co-infection with wild type adenovirus will significantly (5-100 folds) boost transduction efficiency and gene expression level.

**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 3-7 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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## *In-vivo* Infection Protocol:

**The following protocol is given for stereotaxic gene delivery of rAAV to the rodent brain.**

**Materials:**

- Super purified rAAV in PBS, in vivo grade.
- Ethanol 70%.
- Mice or rats.
- Anesthetics and analgesics (e.g., ketamine etc).
- Sterile PBS
- Bone wax
- Triple antibiotic ointment

**Procedures:**

1. Anesthetize and fix animal in stereotaxic apparatus.
2. Make incision and locate bregma.
3. Surgery - Preparation of craniotomy by referring to the standard stereotaxic coordinates of mouse or rats. The craniotomy is drilled using a hand-held drill. The following table is an examples of stereotaxic coordinates.

| Targeted region adult mouse brain00 | Rostral (+) caudal (-)(mm) | Lateral (mm) | Ventral (mm) |
|---|---|---|---|
| Subthalamic nucleus | - 1.9 | 1.6 | 4.4 |
| Dorsal hippocampus, CA1 | - 2.1 | 2.0 | 1.4 |
| Basolateral amygdala | - 1.5 | 2.75 | 4.75 |
| Lateral ventricle | + 0.5 | 0.75 | 2.5 |
| Nucleus accumbens, core | + 1.1 | 1.2 | 4.5 |

4. Injection of rAAV for a single injection. Place the injection micropipette into the holder of the stereotaxic arm. Fill the micropipette with 3 µL rAAV. Then slowly lower the micropipette to the desired z coordinate of the injection site. Apply pressure steadily via a pump or syringe to inject 100~500 nL rAAV at >1E+13 VG/mL within 60~90 seconds.
5. After recovery of animal, feed the animal as usual until the transgene expression is checked 4 weeks after rAAV injection.

**Note:** If rAAV is administrated to mouse via different routes, please refer the rAAV injection dose to the following table.

| rAAV Administration Route | Dose (VG) |
|---|---|
| Intracerebral | $10^9$~$5x10^9$ |
| Intraventricular | $10^9$~$5x10^9$ |
| Intravenous | ~$5x10^{11}$ |
| Intranasal | $10^{10}$~$3x10^{10}$ |
| Intravitreal | $10^9$~$4x10^9$ |