User Manual for AAV Products

Storage and Dilution of AAV

1. AAV virus can be stored at 4°C for a short time (less than a week) before using after the reception. AAV viruses l stock should be stored at - 80°C freezer immediately upon arrival for long-term usage.
2. We suggest redetecting the virus titer before using it if the AAV viruses have been stored for more than 12 months.
3. If desired, aliquot viral stock upon arrival, and store those aliquots at -80°C freezer immediately.
4. Dissolve the virus in ice water if virus dilution is required. After dissolving, mix the virus with sterile PBS or normal saline solution, keeping at 4°C (using within a week).

Note: Avoid repeated freezing and thawing. AAV viruses are sensitive to freeze-thawing, and the titer drops with repeated freeze-thawing

In-vitro Infection Method:

1. Prepare virus-containing media:
   Thaw viral stock at either room temperature or on ice.
   Add the desired amount of virus to growth media to achieve the desired MOI.
   （MOI is a multiplicity of infection, namely the number of virus particles needed for infecting a cell）
   e.g. If you intend to infect 1 million cells using MOI of 1,000 , you need 1,000 x 1,000,000 = 10^9 GC for the infection. If the original stock is 10^{13} VG/mL, you will need 0.1μL of the original stock for the dilution.
2. Infecting cells with the virus:
   Remove the original cell culture media, and add the above AAV-containing media to cell culture.
   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 the infection without causing any undesired effects.

Note:
1. To determine the optimal MOI and virus serotype for your study, you could conduct pilot testing in your cell line using reporter AAV like AAV-GFP.
2. The optimal MOI differs dramatically between cell types for different serotypes of AAV. For example, a range of 1,0000-10,00000 MOI is used for most cell lines for AAV9. Higher MOI may be used for some cells that are difficult to infect.
3. In general, MOI with high transduction efficiency but lowest cell mortality were selected. However, for some cell types, no matter how much MOI is adjusted, high transduction efficiency may not be achieved.
4. Due to the tissue specificity of rAAV, the efficiency of cell infection in vitro is relatively low, so we generally recommend that you purchase rAAV for in-vivo Infection experiments.

Table1. Cell infection efficiency of rAAV in vitro

Cell line	Tissue or cell type	Infectivity of vector:									
		AAV-1	AAV-2	AAV-3	AAV-4	AAV-5	AAV-6	AAV-8	AAV-9	AAV-DJ	AAV-DJ/8
Huh-7	hu liver	4e3	5e2	2e4	2e6	4e5	5e3	7e4	**7e6**	1e2	3e5
293	hu kidney	2e3	5e2	2e4	7e5	4e5	1e4	7e4	**7e5**	1e2	2e5
HeLa	hu cervix	7e4	2e3	1e5	**2e6**	3e4	2e5	1e6	**2e6**	3e2	1e6
HepG2	hu liver	2e6	5e4	3e5	2e7	3e6	1e6	2e7	**ND**	4e3	1e7
Hep1A	mu liver	1e4	2e3	1e6	2e5	2e6	2e5	1e6	**2e7**	5e2	2e6
911	hu retina	6e3	1e3	9e3	5e5	7e5	6e3	1e6	**ND**	2e2	4e5
CHO	ha ovary	1e4	1e4	7e4	7e5	3e3	2e4	1e5	**1e6**	4e1	2e5
COS	si kidney	3e3	1e3	3e3	3e4	2e4	7e3	5e4	**2e5**	2e2	3e5
MeWo	hu skin	2e3	2e2	1e3	7e4	3e3	2e3	2e4	**1e5**	7e0	2e4
NIH3T3	mu fibroblasts	2e5	2e4	7e5	7e5	7e6	2e5	7e6	**ND**	4e3	2e7
A549	hu lung	7e4	1e4	5e4	**ND**	2e6	1e5	2e6	7e6	1e3	2e7
HT1180	hu fibroblasts	5e4	1e4	1e5	7e6	3e6	3e4	2e6	**1e7**	3e3	5e6
Monocytes	hu primary monocytes	9e5	1e7	**ND**	**ND**	8e6	7e5	**ND**	**ND**	1e7	**ND**
Immature DC	hu monocyte-derived DC	8e5	2e7	**ND**	**ND**	9e6	7e5	**ND**	**ND**	1e7	**ND**
Mature DC	hu monocyte-derived DC	9e5	2e7	**ND**	**ND**	6e6	6e5	**ND**	**ND**	2e7	**ND**

（Dirk Grimm, et al. JOURNAL OF VIROLOGY, 2008）

Table2. The general guideline for the Volume of culture medium used

Type	Single-hole bottom area	Recommended volume
96 -well plate	0.3 cm^2	100 μL
24 -well plate	2 cm^2	200~500 μL
6-well plate	10 cm^2	1~2 mL
10cm-plate	60 cm^2	8~12 mL

In-vivo Infection Method:

1. The recommended amount of rAAV in infected mouse tissues injected through the tail vein is 10^{11-12} VG.
2. For mouse brain infection, it is recommended to use 0.2~0.5 μL of rAAV(1.00E+12 VG/ml) .To determine the optimal injection amount for a specific brain area, you could conduct pre-experiments according to the references.

1. Commonly used coordinates for mouse brain stereotaxic injection:

Structure	AP（mm）	ML（mm）	DV（mm）
DG	-1.7	-1	-2
CA1	-1.7	-0.85	-1.5
CA3	-1.7	-2	-2
S1BF	-1.7	-3	-1.5
BLA	-1.4	-3.25	-4.85
MS	0.64	0	-3.8
ACC	1.34	0.4	-2
HDB	0.14	-1.5	-5.5
LH	-1	-1.1	-5
OB	4.3	-1.25	-2