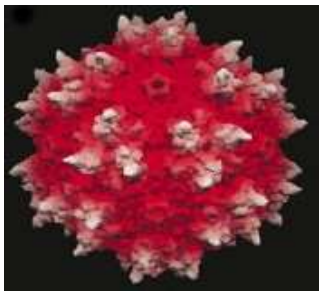
Care and Handling of AAV



It is useful to think of AAV as a very large protein (MW= ~5,200,000). As such it is relatively stable but should be treated with at least as much care as other proteins. It is resistant to most proteases and a variety of chemical treatments that would inactive many viruses. However, because it is so large, it has a large surface area and is more likely to stick to hydrophobic surfaces and aggregate than smaller proteins.

Recombinant AAV can have very different species and cell type specificities ("tropisms") with respect to their infection capabilities. Therefore, it is important to select a serotype that will infect your cells of interest. Also, kinetics of transduction and expression level may vary. One way to determine if an AAV infects your cells is to do a literature search or ask other researchers working in your field which virus is best suited to a particular application. If you are not able to determine if a given virus will infect your cells of interest in this way, then you may have to test the infectivity of your cells. The virus core can provide a wide variety of **stock viruses encoding GFP or Luciferase (Firefly/Renilla) as a reporter** for this purpose.

After virus production, there are different methods for purification which may have certain advantages or disadvantages for your downstream applications. Also, it is essential to discuss buffer conditions that best meet your needs. **Virus production is not the same for every transfer plasmid.** Typically there may be a 10-fold range of virus productivities between various plasmid backbones and transgenes.

Quantification of viruses can be done by different methods – those methods are not comparable so they all need to refer to your own application. Concentrations of viral genomes per ml are not comparable with capsid numbers per ml determined by ELISA/Dot Blot or "infectious titers" determined after transduction of cells. The latter one is an absolutely cell-specific value. Please perform a dilution curve with your cells of interest, then you can always correlate those data with titers based on the same method.

Please discuss these issues with the Core Facility before virus production.

DON'T:

1. **Don’t expose to environmental extremes** (pH, chelating agents like EDTA, temperature, organic solvents, protein denaturants, strong detergents, etc.) that denature proteins.
2. **Don’t introduce air into the sample** by vortexing, blowing bubbles and similar operations (which results in protein denaturation).
3. **Don’t dry** (which results in protein denaturation).
4. **Don’t freeze and thaw multiple times.** AAV is more stable than many viruses or proteins and can be frozen and thawed several times with minimal loss of activity but it is best to avoid.
5. **Don’t expose to “regular” plastics** (especially polystyrene or similar very hydrophobic plastics) for prolonged periods in a liquid phase. Most AAVs are very sticky and losses can occur if they are exposed to regular plastics (e.g., tubes, cell culture plates, pipette tips) if they are not frozen. It is best to store thawed AAV in siliconized or low protein binding tubes and pipette it with similar pipette tips. Pluronic F-68 used at 0.01%-0.1% in the formulation buffer will minimize sticking if regular plastics are used.
6. **Don’t’ dilute into low salt.** Some AAVs (e.g., AAV-2) aggregate in low salt and if the aggregates are large they will be non-infectious.
7. **AAV carry single-**stranded genomes and therefore rely on DNA second strand synthesis in the host cell to become a substrate for host RNA Polymerase. Before transduction remove all reagents that might interfere with the cellular DNA synthesis machinery (e.g. AraC). **Exception**: so-called “self-complementary” AAV carry double-stranded genomes and can be applied directly.

DO:

No addition of glycerol is needed for storage at -80°C, however, repeated freeze-thaw cycles are to be avoided strictly. AAV particles are stable for long time (almost indefinitely) at -80°C. Storage at -20°C is not recommended. At 4°C the virus is stable for several days, but is said to loose about 50% of infectious titer after 1 month. Lyophilization is possible.

NOTE:

1. AAV appears to retain infectious activity for > 10 years when stored at -80C.