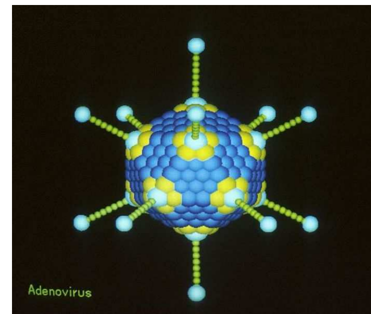
Care and Handling of Adenovirus



It is useful to think of Adenovirus as a very large protein (MW= ~ 150 MDa). 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 Adenovirus Serotype 5 enters cells via the Coxsackie and Adenovirus receptor (CAR) which may be differentially expressed on different cells. One way to determine if Adenovirus 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. This also gives you an idea if toxicity may occur. The virus core can provide a wide variety of **stock viruses encoding GFP or mCherry 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.** Virus in DMEM can endure repeated (more than 30) freezing/thawing cycles without any drop in titer. In the case of CsCl purified virus, a drop of up to 1 log in titer can be observed after storage at -80°C depending on the buffer used. The buffer and pH in particular are critical for preservation of the titer. For optimal stability upon storage, we recommend the following buffer: Tris 10mM, pH 8.0, with 2 mM $MgCl_2$ and 4% sucrose.
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.

DO:

1. The viruses should be stored -80°C especially after purification from culture media (by CsCl usually). In optimal buffer (10 mM Tris HCl pH 8.0, 2 mM $MgCl_2$, 4% sucrose) the virus will be stable for 1-2 years; the virus should also be aliquoted to avoid multiple freeze-thaw cycles. Long-term storage at -20°C is not recommended.
2. Virus is DMEM supplemented with serum needs to be stored the same way as purified particles but is usually much more stable than in buffer. Storage of virus in DMEM with serum at 4°C for up to 1 week to avoid freeze-thaw cycles is acceptable.
3. The best buffer to use is 10 mM Tris pH 8.0, 2 mM $MgCl_2$, 4% sucrose; this buffer enables one to concentrate the virus to approximately 1 x 10^{13} VP/mL (viral particle/mL) without precipitation and provides very good stability for long-term storage and shipping.

   If the virus is to be used for animal studies, a buffer with glycerol should not be used since it is difficult to inject. PBS buffers can also be used but do not provide very good viral stability and should be avoided if the virus has to be concentrated; the particles will likely precipitate due to the low pH (~7) involved. Using a PBS buffer will enable concentration of the virus up to approximately 5 x 10^{11} VP/mL without precipitation. Viruses in PBS buffer will also be severely affected by repeated freeze/thaw cycles. For these reasons we recommend the Tris buffer over PBS for all applications.