User Instructions: AAV for In Vitro Applications

Content

The following table shows the components associated with custom adeno-associated virus (AAV). The specific AAV viral titer is provided in the certificate of analysis (COA) document.

Content	Deliverable	Specification	Recommended use
Pilot-scale packaging	Custom virus	Concentrated virus (>2×10^{11} GC/ml, 10×25 ul)	Cell culture applications
	Control virus	Concentrated virus (>2×10^{11} GC/ml, 1×100 ul)	
Medium-scale packaging	Custom virus	Concentrated virus (>2×10^{11} GC/ml, 10×100ul)	
	Control virus	Concentrated virus (>2×10^{11} GC/ml, 2×100 ul)	
Large-scale packaging	Custom virus	Concentrated virus (>>2×10^{12} GC/ml, 10×100 ul)	
	Control virus	Concentrated virus (>>2×10^{11} GC/ml, 2×100 ul)	

Storage and Handling

1. VectorBuilder's non-ultra-purified AAV is recommended for in vitro applications. Our non-ultra-purified AAV is stored in a Tris-based buffer.
2. Upon receiving, AAV should be stored at -80°C for long term storage (stable for at least 1 year), or -20°C for short term storage (stable for 2~3 weeks).
3. Thaw the vial of AAV on ice prior to use and keep it on ice during the experiment. Thawed AAV can be stored at 4°C for 1~2 weeks without significant loss of biological activity.
4. After thawing, AAV can be dispensed into smaller aliquots according to the quantity used in your experiment and then refrozen. If you need to dilute the virus, you may use PBS, but do so ONLY immediately prior to use.

CAUTION: Do not freeze and thaw your AAV sample multiple times. AAV can be frozen and thawed several times with minimal loss of activity but it is better to avoid this.

Safety Precautions

All AAV viruses from VectorBuilder consist of recombinant transgene sequences flanked by the AAV inverted terminal repeats (ITRs). The AAV ITRs, consisting of only 6% of the wild type AAV genome, are the only AAV specific sequences packaged into the virus particles. The removal of the majority of viral structural genes renders the virus replication defective and dependent on adenovirus helper provided in trans. The recombinant AAV viruses are generated in the presence of a helper plasmid, not helper virus. The viruses are generated by transient transfection of 293T cells using three plasmids (the cis ITR-containing plasmid, the trans plasmid encoding AAV replicase as well as capsid genes, and the adenoviral helper plasmid) which result in the pseudotyping of vector genomes with different serotype capsid proteins. The recombinant AAV viruses are based on wild type AAV virus which is non-pathogenic in human. Although the replication of wild type AAV is dependent on the presence of adenovirus or herpesvirus and will stably integrate into the host cell genome in the absence of helper virus, the recombinant AAV virus genomes remain primarily episomal in target cells and have a low (if any) frequency of integration. **We recommend that the viruses should be handled according to Biosafety Level 2 (BSL-2) criteria.** All handling, storage and disposal of biohazard waste must be in accordance with published and institutional criteria.

Transduction of Target Cells

AAV transduction is cell type-dependent. Some cell types exhibit low transduction efficiency, while others transduce very readily. When designing AAV transduction experiments, it is recommended to use different serotypes of a reporter vector such an AAV expressing eGFP (e.g. VectorBuilder's AAV Serotype Testing Panel) to determine optimal serotype for transduction of your tissue or cell culture. Start transducing the cells at a multiplicity of infection (MOI) between 1x10^{4} and 1x10^{6} genome copy (GC) per cell if the cells are readily transducible. With some cell lines a higher MOI might be needed. Look for the highest transduction with minimal cell death. With some cell lines, high transduction levels cannot be achieved.

Protocol for transducing mammalian cell line:

1. Day before transduction (Day 0)
   Plate target cells in appropriate medium so that they will be 30~50% confluent at the time of transduction. Incubate 18-20 hours at 37 °C in a humidified 5% CO_2 incubator. For example, when using 293T cells, we usually plate $3x10^5$ cells per well in a 6-well plate.
2. Day of transduction (Day 1)
   - Thaw virus on ice. Take the appropriate amount of virus as needed to achieve the desired MOI, place in an appropriate amount of medium, and mix gently (but do not vortex). To maximize transduction efficiency, use the minimum amount of medium necessary to cover the surface of the plate. For example, when infection is performed in 6-well plates, we use 1 ml of medium per well.
   - Aspirate old medium from target cells, then add the virus-containing medium onto the cells.
   - Swirl the plate gently to mix and cover the cells. Incubate at 37 °C in a humidified 5% CO_2 incubator overnight.

     Note: If you are concerned that exposure to the viral supernatant may adversely affect the target cells, limit the transduction to 6-8 hours.
3. Day 2
   Remove the virus-containing medium, and replace with fresh complete culture medium. Incubate at 37 °C in a humidified 5% CO_2 incubator overnight.
4. Day 3 and onward
   Analyze gene expression at desired time points following viral infection. In general, detectable levels of your gene product should be evident 24-48 hours after transduction.

   Note: In actively dividing cells (i.e. doubling time of approximately 24 hours), transgene expression is generally detectable within 24 hours of transduction, with maximal expression observed at 48-96 hours (2-4 days) post-transduction. Expression levels generally start to decline 5 days post-transduction. In cell lines that exhibit longer doubling times or non-dividing cell lines, high levels of transgene expression normally persist for a longer time. If you are transducing AAV into your mammalian cell line for the first time, we recommend performing a time course study to determine the optimal temporal conditions for expression of your transgene.

Example of Anticipated Results

An example of successful transduction is shown in Figure 1.

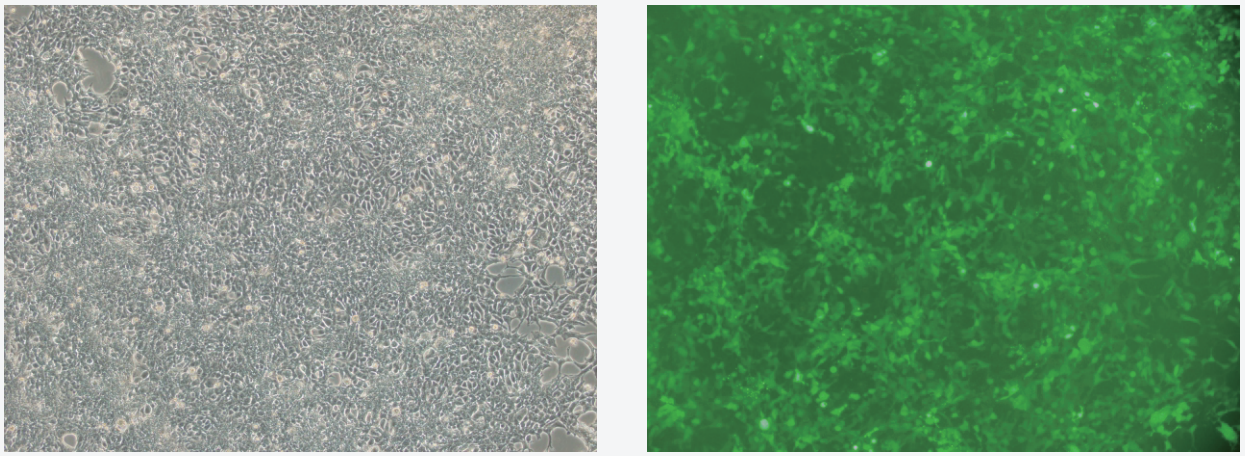

Figure 1. EGFP expressing AAV2 was used to transduce 293T cells at MOI 10000 using the protocol in this document. Images were taken at 100X. Left: bright field; right: EGFP.