User Instructions: AAV Serotype Testing Panel

Content

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

Product Name	Catalog No.	Titer & Volume
In vitro grade AAV serotype testing panel (CMV-EGFP)	PANEL-AAVS01	~10^{12} GC/ml, 25 ul
In vitro grade AAV serotype testing panel (CAG-EGFP)	PANEL-AAVS02	
In vivo grade AAV serotype testing panel (CMV-EGFP)	PANEL-AAVSP01	~10^{13} GC/ml, 25 ul
In vivo grade AAV serotype testing panel (CAG-EGFP)	PANEL-AAVSP02	

Notes:

a. **Our standard AAV serotype collection includes AAVs 1, 2, 3, 4, 5, 6, 6.2, 7, 8, 9, rh10, DJ, DJ/8, PHP.eB, PHP.S, AAV2-retro, AAV2-QuadYF and AAV2.7m8.**

b. **The AAVs packaged in this offering are ssAAVs.**

Storage and Handling

1. VectorBuilder's non-ultra-purified AAV is recommended for in vitro applications and is stored in a Tris-based buffer. VectorBuilder's ultra-purified AAV is recommended for in vivo applications and is stored in a PBS-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 vials of AAV on ice prior to use and keep 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 the helper adenovirus provided in trans.

VectorBuilder's recombinant AAV viruses are generated using 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 results in the pseudotyping of vector genomes with different serotype capsid proteins. Recombinant AAV viruses are based on wild-type AAV viruses which are non-pathogenic in human. Replication of wild-type AAV is dependent on the presence of helper adenovirus or herpes virus, which leads to the potential for genomic integration, however, recombinant AAV genomes primarily remain episomal. 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.

AAV Serotype Testing Panel for In Vitro Applications

Transduction of target cells

AAV transduction is cell type-dependent. Some cell types exhibit low transduction efficiency, while others transduce very readily. VectorBuilder's AAV Serotype Testing Panel can be used to determine the optimal serotype for transduction of your 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 the appropriate medium so that they will be 30~50% confluent at the time of transduction. Plate individual wells for each of the serotypes that will be tested. Incubate for 18~20 hours at 37 °C in a humidified 5% CO2 incubator. For example, when using 293T cells, we usually plate $3x10^5$ cells per well in a 6-well plate for a single serotype.

2. Day of transduction (Day 1)

   - Thaw each virus on ice. Pipette 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 it with the fresh complete culture medium. Incubate at 37 °C in a humidified 5% CO_2 incubator overnight.

4. Day 3 and onward

   Analyze EGFP expression in different wells at desired time points following viral transduction, ensuring the microscope's fluorescence intensity is maintained across samples to determine which serotype exhibits the highest fluorescence. In general, detectable levels of EGFP should be evident 24~48 hours after transduction.

   Note: In actively dividing cells (i.e. doubling time of approximately 24 hours), EGFP 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 conditions for future target gene expression.

Example of Anticipated Results

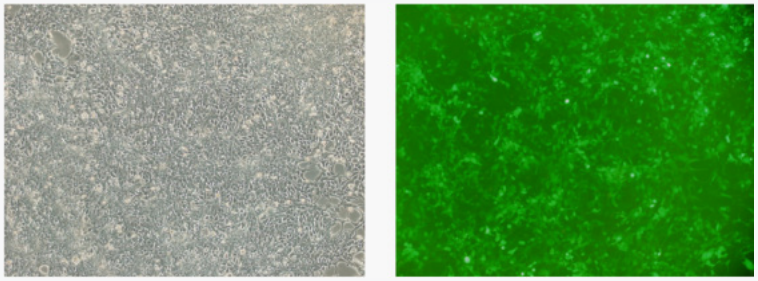

Figure 1. EGFP expressing AAV2 was used to transduce 293T cells at MOI 10,000 using the protocol in this document. Images were taken at 100X. Left: bright field; right: EGFP.

AAV Serotype Testing Panel for In Vivo Applications

Recommended tissue tropism of AAV serotypes

Tissue Type	Recommended AAV Serotypes
Smooth muscle	AAV1, AAV2, AAV3, AAV5, AAV6, AAV7, AAV8, AAV9, AAV-rh10
Skeletal muscle	AAV1, AAV9
CNS	AAV1, AAV2, AAV4, AAV5, AAV7, AAV8, AAV9, AAV-rh10, AAV-PHP.eB
PNS	AAV-PHP.S
Brain	AAV1, AAV2, AAV5, AAV7, AAV8, AAV-DJ/8
Retina	AAV1, AAV2, AAV4, AAV5, AAV7, AAV8, AAV9, AAV-rh10, AAV2-QuadYF, AAV2.7m8
Inner ear	AAV1, AAV2, AAV6.2, AAV8, AAV9, AAV2.7m8
Lung	AAV1, AAV3, AAV4, AAV5, AAV6, AAV6.2, AAV9, AAV-rh10
Liver	AAV1, AAV2, AAV3, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAV-rh10, AAV-DJ, AAV-DJ/8
Pancreas	AAV1, AAV2, AAV6, AAV8, AAV9, AAV-rh10
Heart	AAV1,AAV4, AAV5, AAV6, AAV8, AAV9, AAV-rh10, AAV-DJ
Kidney	AAV2, AAV4, AAV8, AAV9, AAV-rh10, AAV-DJ, AAV-DJ/8
Adipose	AAV6, AAV8, AAV9
Testes	AAV2, AAV9
Spleen	AAV-DJ, AAV-DJ/8
Spinal nerves	AAV2-retro
Endothelial cells	AAV2-QuadYF

Note: The ITRs carrying your gene of interest (GOI) are from the AAV2 genome. Different serotypes are distinguished by the capsid protein.

Recommended Volume for Mouse In Vivo Injection

To transduce ultra-purified AAV to mouse tissues successfully, the following table lists the recommended injecting volumes for different injection methods.

Injection Sites	Recommended Volume
Lateral ventricles	5 ul
Nucleus accumbens	0.05~0.1 ul
Ventral tegmental area	
Hippocampus	
Jugular vein	≥100~250 ul
Tail vein	100~250 ul
Lung	10 ul/site, 5 sites in total
Abdominal cavity	200~250 ul
Vitreous humor	1~2 ul
Heart	1.5~3 ul/site, 5 sites in total
Muscle	10 ul/site, 4 sites in total

Note: For all tissues, the expression of the transgene carried by AAV can last for around 2~6 months. Usually, the peak expression can be detected at 2~4 weeks post-injection.

For detailed protocols on mouse tail vein injection and mouse intra-cerebroventricular injection, please visit our user instructions for AAV in vivo application.