AAV Vectors: General Information

AAV vectors contain recombinant transgene sequences (e.g. encoding reporter or therapeutic genes) 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 vector particles. The removal of the viral structural genes renders the vector replication-defective and dependent on adenovirus helper functions provided in trans. AAV vectors produced by the Penn Vector Core are generated in the presence of a helper plasmid, not helper virus. The vectors are generated by transient transfection of HEK293 cells using three plasmids (the cis ITR-containing plasmid, the trans plasmid encoding AAV replicase and capsid genes and the adenoviral helper plasmid) which result in the pseudotyping of vector genomes with different serotype capsid proteins. The recombinant vectors are purified by tangential flow filtration followed by iodixanol gradient purification and buffer exchange. Routine quality control conducted for preclinical vector preparations includes a determination of titer and yield by quantitative PCR and endotoxin analysis. Additional assays may include a purity assessment by SDS-PAGE/densitometry and/or an infectious titer determination by TCID₅₀ analysis. Assays for the detection of replication competent (RCAAV) AAV particles are not currently available for serotypes other than AAV2 and not conducted for preclinical vectors.

AAV vectors are based on AAV viruses which are non-pathogenic in humans and the vectors themselves are not known to cause any diseases in humans or animals. Although wild type AAV virus is dependent for replication on the presence of adenovirus or herpesvirus and will, in the absence of helper virus, stably integrate into the host cell genome, AAV vector genomes remain primarily episomal in target cells and have a low (if any) frequency of integration. Cultures of replication defective AAV vectors are non-infectious and are not hazardous materials as defined by OSHA 1919.1200. The NIH Guidelines state that adeno-associated virus (AAV) types 1 through 4, and all recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus (which is the case at UPenn) can in most cases be handled at biosafety level 1 (BSL-1). This level of containment made is modified by other considerations.

AAV vectors typically fall into rDNA registration category Class III-D (experiments that require institutional biosafety committee approval before initiation). If the vectors are to be used for in vivo studies, registration Class III-D-3 (experiments involving whole animals) could be appropriate. If the vectors are designed to express cDNA from higher risk group organisms (e.g HIV), they would move to a Class III-D-2. In terms of biosafety containment level, UPenn requests investigators to state if the vectors are to be registered for generation, for use, or both. BSL-2 conditions must be used for the generation of AAV vectors due to the transformed HEK293 cells that are used for production. Purified AAV vectors may be subsequently used under BLS-1 conditions. Additional information is provided in the attached MSDS. It is important to note that vectors are different from the viruses from which they were derived but some of the safety information may be the same as required for the wild type virus.
Replication defective recombinant AAV vectors are non-infectious and non-hazardous materials as defined by OSHA 1910.1200. The vectors may be used under biosafety level (BSL)-1 conditions however they are generated under BSL-2 conditions due to the use of HEK293 cells in the production process. Routine quality control on preclinical vectors includes determination of yield and endotoxin contamination however the vectors are not tested for cellular contaminants or for replication competent AAV so should be handled as potentially infectious material.

SECTION I - IDENTITY
NAME: Recombinant adeno-associated viral vector
SYNONYM OR CROSS REFERENCE: AAV vector, rAAV
CHARACTERISTICS: Parvovirus; non-enveloped, 20-26 nm diameter, linear DNA genome
PROVIDED: Frozen suspension of rAAV particles in sterile PBS with 5% glycerol

SECTION II - HEALTH HAZARD
PATHOGENICITY: None. AAV is not known to cause any diseases in humans or animals
HAZARDOUS INGREDIENTS: None

SECTION III - VIABILITY
DRUG SUSCEPTIBILITY: No specific antiviral available
SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 10% chlorine bleach (recommended)

SECTION IV - RECOMMENDED PRECAUTIONS
CONTAINMENT REQUIREMENTS: Appropriate containment facilities for all activities involving the vector and vector-administered cells, tissues and fluids. This includes BSL-2 practices for rAAV vector generation and BSL-1 practices for rAAV vector use (including animal housing).
PROTECTIVE CLOTHING: Laboratory coat, gloves, safety glasses recommended

SECTION V - HANDLING INFORMATION
SPILLS: Allow aerosols to settle; contain spill and decontaminate with 10% chlorine bleach; allow sufficient contact time (30 min) before clean up
DISPOSAL: Decontaminate all wastes before disposal: steam sterilization, chemical disinfection with 10% chlorine bleach (liquid wastes), incineration (tissues or animal carcasses)
STORAGE: In sealed containers that are appropriately labeled. Long-term storage at -80°C

For information on BSL-2 handling, see Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition The above information is accurate to the best of our knowledge and experience. The user should exercise independent judgment as to the hazards based on all sources of information available. For current MSDS information, please visit the Penn Vector Core website.