VIRAL VECTOR REFERENCE GUIDE

AUBURN

UNIVERSITY

ADENO ASSOCIATED VIRUS & RECOMBINANT ADENO ASSOCIATED VIRUS (AAV/rAAV)

Table of Contents

BACKGROUND

Adeno-associated virus (AAV) and recombinant adeno-associated virus (rAAV) are commonly used for gene expression with fewer associated biosafety concerns when compared to viral vectors that are persistent and able to integrate into the genome. Adeno-associated viruses are non-enveloped, single-stranded, DNA viruses belonging to the parvovirus family. The AAV can only replicate in the presence of a helper virus such as adenovirus (Ad), herpes simplex virus, human papillomavirus, or vaccinia for productive infection.

Recombinant AAV-vectors of various serotypes are widely used for gene delivery because of their characteristics, the ability to infect a broad range of cells, the limited cloning capacity, and the stable expression in a pH range of 3 to 9 and up to 56°C.

DIFFERENT AAV SEROTYPES

The following table lists various AAV serotypes, and their specific tissue targets.
IBC requires which serotype(s) will be used for research.

TISSUE TARGET	RECOMMENDED SEROTYPES
Central Nervous System	AAV1, AAV2, AAV5, AAV8, AAV9, AAV-DJ, AAV-DJ9
Cardiac	AAV1, AAV8, AAV9, AAV-DJ9
Liver	AAV8, AAV-DJ
Lung	AAV6, AAV9
Kidney	AAV2
Pancreas	AAV8
Photoreceptor	AAV2, AAV5, AAV8
RPE	AAV1, AAV5, AAV8
Skeletal Muscle	AAV1, AAV6, AAV8, AAV9

SOURCE : https://www.vectorbiolabs.com/adenovirus-vs-aav/

NIH GUIDELINES

The NIH Guidelines for Research Involving Recombinant or Synthetic DNA Molecules (NIH Guidelines) identify all AAV serotypes and recombinant or synthetic AAV constructs as risk group 1 (RG1) agents so long as the transgene:

1. Does not encode a potentially tumorigenic gene product (e.g., oncogene).
2. Does not lead to production of a toxin molecule.
3. Is produced in the absence of a helper virus.

If the vector transgene encodes for a potentially toxic or tumorigenic gene the risk group changes accordingly. (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf)
*Please see below sections on supporting information required for BUA submission.

AAV EXPERIMENTS DESIGNATED AS BSL/ABSL-1 CONTAINMENT

BSL1/ABSL-1 containment will be considered only **if all three requirements** are met:

1. Transgene does not express an oncogenic protein or toxin.
2. AAV/rAAV is generated without using adenovirus or any other helper virus of human origin.
3. AAV/rAAV is propagated in insect cell lines or is purified sufficiently before use if using HEL293 or HEK393T cells or other cell lines.

If purified, a description of purification procedures (e.g., iodixanol gradient, column chromatography, etc.) and/or documentation from the source vector core facility that describes purification procedures must be included. **This needs to be documented in the BUA.**

Recognized AAV production Core Facilities

Core	Purification Procedure	BSL
UNC	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE/Silver Stain per vector per lot Will provide purity and titer per lot	Automatic BSL-1
MWRI	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE/Silver Stain per vector per lot Will provide purity and titer per lot	Automatic BSL-1
Addgene	Iodoxinal Gradient followed by concentration QC analysis by qPCR titer, SDS-PAGE/Silver Stain Will provide results of QC upon request	Automatic BSL-1
Salk Institute (CA)	Purification on a discontinous OptiprepTM gradient; price per prep. Custom rAAV preps are titrated using qPCR to give titer in genome copies (GC) per ml	BSL-2 unless purification and QC data provided
Stanford	Provides unpurified AAV unless otherwise requested Core facility recommends use under BSL-2	BSL-2 unless purification and QC data provided
U Penn	Iodoxinal Gradient + Column Purification QC analysis by SDS-PAGE is available upon request Will provide purity and titer per lot at cost	BSL-2; downgrade possible with QC data provided

Source: University of Pittsburgh IBC guidance on AAV

RESEARCHERS CONSTRUCTING THEIR OWN RECOMBINANT AAV

Researchers constructing their own recombinant AAV must provide:

- The type and source of cell lines used.
- The exact cassette which is going to be constructed. If multiple, please list all.
- The method of purification used in the lab.
- The method and results/proof of the QC of purification performed.
    - If using human cell lines, researchers must ensure no contaminants are present.
- Documentation of purification during BUA approval and during post approval monitoring/annual lab inspections.

BSL2/ABSL2 CONTAINMENT

If the above conditions are not met, BSL2/ABSL2 containment is required when working with AAV and rAAV. **The protocols will be reviewed on a case-by-case basis.**

A few rationales for requiring BSL2/ABSL 2 containment include:

- Whether any possible health hazards which may be associated with using wild type AAV as well as using any helper virus. (This will be a major concern for human gene therapy trials.)

- The AAV vectors are not sufficiently purified as mentioned in Section 4, it can contribute to residual human cell contaminants if the propagation has taken place using HEL293 or HEK393T (human embryonic kidney continuous cell line) cells or other cell lines either in the presence of a helper virus or by co-transfection of helper plasmids. Due to the possibility of human cell contaminants and following the Auburn Biosafety Manual - https://cws.auburn.edu/shared/files?id=227&filename=bsm2.pdf, any Use of Human Cell Lines requires handling in accordance with the OSHA Bloodborne Pathogens Standard (29 CFR 1910-1030) under BSL2 containment. This includes well established human cell lines.

- The nature of transgene expression results in expression of an oncogene protein or toxin.

- The vector is generated using wild type adenovirus or any other helper virus of human origin.

- The AAV vectors (by description) to be used in the laboratory are usually handled at BSL2 containment.

BRIEF SYNOPSIS OF IBC GUIDANCE

LABORATORY HAZARDS	• AAV/ rAAV is transmitted through direct or indirect contact via aerosol, droplet exposure to the mucous membrane, ingestion, and injection. • Laboratory acquired infections are possible through exposure to lab cultures of wildtype AAV or recombinant viruses. • In the case of an exposure, seek medical attention care as outlined in AU exposure control plan. https://cws.auburn.edu/shared/files?id=227&filename=Exposure%20Control%20Plan%202016.pdf
LABORATORY PRACTICES	The rAAV vectors are considered Risk Group 1 (RG1) agents in accordance with the NIH Guidelines for Research Involving Recombinant DNA Molecules due to the lack of helper viruses in the manufacturing process. If the vector transgene encodes for a potentially toxic or tumorigenic gene, the risk group changes accordingly (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf) The following lab practices are followed. • All manipulations of AAV/rAAV that may generate aerosols, including pipetting, infection of cell culture, infection of animals, and harvesting infected cells, must take place in a biological safety cabinet (BSC). • Display biohazard signs and labels in areas and on equipment where AAV/rAAV is used or stored for hazard communication. • Aerosol containment devices, like sealed rotors or safety cups, must be used when centrifuging. Rotos, cups, and centrifuge tubes should be opened inside the BSC. • Laboratory waste must be decontaminated/autoclaved before being managed like solid waste
ANIMAL BIOSAFETY CONTAINMENT AND WASTE DISPOSAL:	• Adenoviral vector must be administered under ABSL-2 containment with the use of a BSC. • For BSL/ABSL 1 projects, bedding and animal cages can be treated as regular trash without any decontamination. • For projects which are assigned BSL/ABSL2 where animals (including rats and mice) may shed/excrete adenovirus for some time post-administration, animals must be housed under ABSL-2 conditions for 72 hours, after which animals may be moved to ABSL-1 housing. **DECONTAMINATION AND WASTE DISPOSAL for ABSL2:** • During initial 72h, all bedding, and cages must to be decontaminated by autoclaving at 121°C at 15psi for 15mins prior to disposal via regular trash stream. • After 72h, any waste generated can be discarded as regular trash without any decontamination.

DECONTAMINATION IN LABS	Use one of the following disinfection methods against AAV: • Autoclaving for 30 minutes – 1 hour at 121°C under 15 psi of steam pressure • 10% dilution of fresh sodium hypochlorite (bleach) with 10 minutes contact time. • Alkaline solutions with pH >9 or 5% phenol. If using phenol for decontamination, autoclaving the waste is prohibited
TRANSPORTATION	All AAV materials must be properly contained in a closed leak-proof container and labeled biohazard for transport within the university. For shipping outside the university, contact RMS (Tom Hodges or Deepika Suresh) for Material Transfer Agreement.
SUPPORTING INFORMATION REQUIRED FOR BUA SUBMISSION	• A description of purification procedures and/or documentation from the source vector core facility that describes purification procedures. (Refer to Section 4) • Full description of the AAV cassette along with specific serotype with tissue target (Refer to Section 2) to be used. (Hosts, vectors, and gene(s) of interest) • IACUC congruity for all the animal experiments. (Amendment with full committee approval will be required)

SUMMARY TABLE OF BIOSAFETY LEVEL REQUIREMENTS

Biosafety Levels are determined using this chart below;

Summary of biosafety level requirements for AAV/rAAV use				
Oncogene or Toxin	Human origin Helper Virus is used (e.g. human adenoviruses and herpesviruses)	Propagated in Human Cell Lines (e.g. HEK 293 cells)	**Purification and Quality Control	Recommended BSL/ABSL
Yes	Yes	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
	No	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
No	Yes	Yes	Yes	2
			No	2
		No	Yes	2
			No	2
	No	Yes	Yes	1
			No	2
		No	Yes	1
			No	1

SOURCE: UNIVERISTY OF PITTSBURG IBC GUIDANCE ON AAV

REFERENCES

Dismuke DJ, Tenenbaum L, Samulski RJ. Biosafety of recombinant adeno-associated virus vectors. *Curr Gene Ther*. 2013;13(6):434-452. doi:10.2174/15665232113136660007