UC RIVERSIDE | Environmental Health & Safety

Lentiviral Vectors

Standard Operating Procedure

All research involving the materials described in this SOP must be documented and approved in a Biological Use Authorization (BUA) prior to use.

Containment Level

☐ **BSL-2** ☐ **ABSL-2**
☐ **BSL-2+** ☐ **ABSL-2+**

Principal Investigator: Click or tap here to enter text. Click or tap here to enter text.

BUA #: 20180032.

BUA Expiration Date: Click or tap here to enter text. Click or tap here to enter text.

Type of Lentiviral Vector: Click or tap here to enter text. Click or tap here to enter text.

Type of Insert: Click or tap here to enter text.

Description

Lentiviruses are a subclass of retroviruses which are able to infect both proliferating & non-proliferating cells. Lentivirus vectors systems can include viruses of non-human/non-primate origin (Feline Immunodeficiency Virus (FIV), Equine Infectious Anemia Virus (EIAV)) as well as simian viruses (Simian Immunodeficiency Virus (SIV)) or human viruses (Human Immunodeficiency Virus (HIV)). One of the mostly utilized lentiviral vector is based on HIV, which is the virus responsible for the development of Acquired Immunodeficiency Syndrome (AIDS). Lentiviral vectors are modified to provide a safer version of the wild-type virus in which the viral replication genes have been removed. During infection, there is a possibility that the lentivirus may convert to a replication competent state. Although this scenario is highly unlikely, monitoring for such a possibility is encouraged, since such a conversion could compromise laboratory safety.

The main risks for research with lentiviral vectors are the potential for generation of replication-competent lentivirus (RCL) and the potential for oncogenesis via random chromosomal integration. The nature of the vector system (and its safety features) and the nature of the transgene insert encoded by the vector (e.g., expression of a known oncogene with a

constitutive strong promoter may require heightened safety precautions) must also be considered.

The potential for generation of RCL from HIV-1 based lentiviral vectors depends upon several parameters, the most important of which are the number of recombination events necessary to reassemble a replication competent virus genome and the number of essential genes that have been deleted from the vector/packaging system. Based on this, later generation lentiviral vector systems are likely to provide a greater margin of personal and public safety than earlier vectors, because they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein. (It should be noted, however, that pseudotyping with coat proteins such as VSV-G may broaden the host cell and tissue tropism of lentivirus vectors, which will be considered in the overall safety assessment by the IBC). Later generation vector systems also separate vector and packaging functions onto three or four plasmids and they include additional safety features such as the deletion of Tat, which is essential for replication of wild-type HIV-1, and altered 3' LTR that renders the vector "self-inactivating" (SIN). In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCL.

Modes of Transmission

Routes of exposure for lentiviral vectors include percutaneous via sharps (needle-sticks, cuts, or bites), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another route may include inhalation via aerosols generated by the use of equipment such as centrifuges or vortex mixers.

Personal Protective Equipment

- Closed-toe shoes; long pants or equivalent
- Long-sleeved lab coat
- Gloves (double gloves if BSL-2+)
- Face protection when a splash risk is present
- Respirator in some cases if aerosol risk is present

Engineering Controls

- All work with lentiviral vectors must be performed in a BSL-2 or ABSL-2 facility.
- Certified Class II biosafety cabinet (BSC) or other primary containment device is required for all procedures involving the manipulation of lentiviral vectors.
- Replace glass with plastic where possible.

- Engineered sharps protection such as self-sheathing needles are strongly recommended.
- Vacuum lines for aspiration must be equipped with an in-line HEPA filter.
- Centrifuge rotors and buckets should be equipped with aerosol-tight safety covers or buckets.

Procedures

USE.

The practices and procedures defined in the UCR Exposure Control Plan should be followed. All lentiviral vectors should be handled at BSL-2 or ABSL-2. If the insert is an oncogene or involves deletion of tumor suppressor gene, it should be handled at BSL-2+ or ABSL-2+.

- Always wash hands after removing gloves and before exiting the lab.
- Minimize sharps usage.
- Perform procedures within biosafety cabinets and minimize the creation of splashes and aerosols.
- All work surfaces should be decontaminated before and after use using 10% bleach followed by 70% alcohol or Institutional Biosafety Committee (IBC) approved disinfectant.
- Affix a biohazard symbol sticker on equipment used with these materials (i.e. BSCs, centrifuges, incubators, etc.)

CENTRIFUGATION.

Place a biosafety hazard sticker on any centrifuge used for lentiviral vectors. Always use sealed rotors, safety cups, or safety buckets if they are available. Load and unload rotors inside biosafety cabinet. Wipe or spray the exterior of the tubes with approved disinfectant when loading or removing samples from rotor.

If a spill occurs during centrifugation, stop the centrifuge, notify lab members, and leave the lab. Close the door and post a warning sign not to enter. Wait for at least 30 minutes to allow aerosols to settle. Upon return, follow spill procedures to clean up the interior of the centrifuge. All contaminated PPE and cleanup material should be disposed of as biohazardous waste.

STORAGE.

Equipment housing long-term storage of lentiviral vectors (e.g. N_2 dewar or -80°C freezer) should have a biohazard symbol sticker located on the outside (EH&S can provide stickers). Short-term storage of lentiviral vectors should be clearly marked and labeled with the hazard.

TRANSPORT.

All biohazardous material should be transported in a leak-proof primary container labeled with the hazard name then placed into a leak-proof, rigid, non-breakable secondary container clearly labeled with the biohazard symbol as well as PI name and contact information. The secondary container should also contain enough absorbent material to soak up any spill. Transport of lentiviral vectors to vivaria locations must be approved by the IACUC and be reflected in the PI's Animal User Protocol (AUP).

Decontamination and Waste

- All work surfaces should be decontaminated with freshly-made 10% bleach for 5 minutes or approved disinfectant for appropriate contact time.
- Full-strength bleach should be added to liquid waste to achieve a final concentration of 10% bleach. After 30 minutes contact time with the bleach, the liquid waste may be poured down the drain followed by copious amounts of water.
- Gloves and other contaminated solids must be disposed of as biohazardous waste.
- Animal bedding and waste potentially contaminated with lentiviral vectors must be disposed of as biohazardous waste.
- Contaminated sharps should be placed into a red biological sharps container. **Do not recap needles**. Contact EH&S if you require a sharps container (x2-5528 or ehslaboratory@ucr.edu)

Spills

If the spill is inside a Biosafety Cabinet, do not turn off biosafety cabinet or close sash.

Remove any contaminated clothing or PPE and dispose of as biohazardous waste.

Put on fresh gloves (and PPE, if necessary) and cover spill area with paper towels. Pour freshly made 10% bleach (or approved disinfectant) over paper towels starting at the perimeter of the spill moving toward the center; be careful not to splash bleach. Any objects in spill area should be decontaminated with 10% bleach (or approved disinfectant) as well. Allow 30 minutes of contact time for the bleach.

If there are any sharps, including broken glass, use forceps or tongs to pick up and place into sharps container. Place disinfectant-soaked paper-towels into biohazardous waste bag and wipe area with disinfectant and paper towels again. Mop if needed. Remove gloves and dispose as biohazardous waste.

All spills must be reported to EH&S and lab supervisor within 24 hours.

<u>EH&S contact information:</u>

Phone: (951) 827-5528

Email: ehsbiosafety@ucr.edu

Website: https://ehs.ucr.edu/ ('Report an Incident' link at top of page)

First Aid & Emergencies

Workers exposed to bloodborne pathogens and other potentially infectious materials should be evaluated by a medical professional following any exposure incident.

SKIN.

Wash with soap and water for 15 minutes. Carefully remove any contaminated clothing and dispose of as biohazardous waste. Report incident to supervisor and EH&S. Seek medical attention if needed.

NEEDLESTICK / SHARPS INJURY.

Flush wound with soap and water. Immediately seek medical attention. Report incident to supervisor and EH&S.

INGESTION.

Immediately seek medical attention. Report incident to supervisor and EH&S.

MUCOUS MEMBRANE.

Flush at emergency eyewash station for at least 15 minutes. Ask for assistance, if necessary. Seek immediate medical attention. Report incident to supervisor and EH&S.

All exposures must be reported to EH&S and lab supervisor within 72 hours.

EH&S contact information:

Phone: (951) 827-5528

Email: ehsbiosafety@ucr.edu

Website: https://ehs.ucr.edu/ ('Report an Incident' link at top of page)

References

UCR Bloodborne Pathogens and Aerosol Transmissible Diseases Exposure Control Plan
https://ehs.ucr.edu/sites/g/files/rcwecm1061/files/2020-06/BLOODBORNE%20PATHOGENS%20AND%20AEROSOL%20TRANSMISSIBLE%20DISEASES%2006-2020.pdf

BMBL 6th Edition
https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

Addgene Lentiviral Guide
https://www.addgene.org/guides/lentivirus/

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Acknowledgement

By signing below I acknowledge that I have read, understand, and agree to abide by the procedures and practices described in this document.

_____ _____

Principal Investigator **Date**

Name	Signature	Date