

PI Name:

Safety Protocol for working with Baculovirus

The MUSC Institutional Biosafety Committee requires Biosafety Level 2 (BSL-2) containment and safety practices for work involving Baculovirus.

1. Hazard Communication Statement

Baculovirus is a lytic virus, primarily pathogenic for insects. Baculovirus vector systems are often used to obtain a high level of expression of a desired protein in insect cells (Sf9 cells). In the natural environment, wild type baculovirus can pose a threat to certain insect species; however, commonly used baculovirus based vectors have been modified to reduce the pathogenicity to insects.

Transmission of baculovirus is through direct contact with the infective virus/vector. Baculovirus is highly sensitive to human complement and, therefore, should an exposure occur, rapid inactivation of the virus is anticipated. The budded form of the virus routinely used in research is non-infectious for the insect host, decreasing the risk to the environment should accidental release occur. Polyhedrin negative baculovirus expression systems are susceptible to desiccation and UV light limiting survival time to hours. Wildtype and AcNPV baculovirus with normal polyhedron genes survive for days or weeks in the environment.

Generally, non-genetically modified wild-type baculoviruses cannot replicate in vertebrate cells and thus have minimal but not, non-existent risk to laboratory personnel. However, more recent studies with the use of mammalian specific promoters have achieved expression of foreign genes in a wide variety of mammalian cell lines and primary cell cultures. Thus, while baculovirus is mainly infective for insect cells, some recombinant baculovirus vectors can infect mammalian cells and should therefore be handled under BL2 conditions.

2. Laboratory Precautions

1. Standard Laboratory practices

Viruses shall be handled with appropriate precautions consisting primarily of **good microbiological laboratory techniques** as well as Biosafety Level 2 (BSL-2) containment. The following precautions should be employed:

- A. Access to the laboratory is limited or restricted at the discretion of the laboratory director.
- B. Placards should be placed on the entrances to the lab listing biological hazards and the PI's name and 24/7 contact information for the PI and/or laboratory personnel familiar with the biohazard.
- C. Do not store food in lab.
- D. Do not eat, drink, smoke, handle contact lenses, apply cosmetics (including chap stick), etc. in the lab.
- E. Do not mouth pipette.
- F. Plants or animals not involved in experiments are not allowed in the lab.
- G. Laboratory personnel must be appropriately trained.
- H. The safety protocol (SOP) serves as training documentation and reference information. A copy signed by laboratory personnel should be stored in the lab's safety manual.
- I. Vacuum lines must be HEPA filtered
- J. Liquids should be handled carefully to minimize creation of splashes and aerosols. Centrifugation should be performed using sealed tubes and sealed rotors or safety cups.
- K. Sharps should be handled with extreme caution to avoid cuts or autoinoculation during use and disposal. Needles should not be bent, sheared, or recapped. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, by autoclaving or incineration.
- L. Transport: Infectious or biohazardous materials must be transported in a sealed primary container inside a sealed durable and leak proof secondary container that has been labeled with a biohazard sticker
- M. Lab personnel must wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory or animal facility.

2. Personal Protective Equipment (PPE)

- A. Wear protective gear including disposable gloves and a cloth or disposable lab coat.
 - If using a cloth lab coat, it must remain in the cell culture room, virus procedure room or inside a biohazard bag in the lab.
- B. Wear safety glasses and face protection when splashes, sprays or aerosols can be expected.
- C. Dispose of contaminated PPE in biohazard bags/containers.
- D. No personal protective equipment shall be worn outside of the lab.

3. Working Procedures for Viral Vectors

Before working with virus:

- prepare a solution of 10% bleach in water in appropriate containers for disinfecting supplies that may come in contact with the virus. This solution should at minimum be prepared fresh weekly.
- put on PPE

While working with virus:

- always use aseptic technique
- avoid the spread of contamination and immediately replace gloves, if contamination is suspected.

After working with virus:

- If applicable, vacuum lines will be rinsed with 10% bleach.
- Treat liquid waste with bleach to a final concentration of 10% bleach for a minimum contact time of 30 minutes. After 30 minutes, dispose of liquid waste in a sink with copious amounts of running water.
- Solid waste including pipettes, containers, etc. that come in contact with virus must be disinfected with 10% bleach prior to disposal in biohazard waste containers. Autoclave biohazard waste upon completion. Dispose of solid waste in orange bags, which are autoclaved and placed in a red biohazard bag/container for final disposal.
- Following disposal of the liquid waste, rinse glassware with 10% bleach, followed by washing and autoclaving.
- Disinfect work surfaces with 10% bleach. Bleach can corrode metal surfaces, but this can be avoided by wiping the surface with water or 70% ethanol after decontaminating to remove the bleach.
- Remove disposable PPE and dispose of it in biohazard bags.
- Wash hands with soap and water.

3. Emergency procedures

3A. Spills of virus:

- 1) Notify workers in the area.
- 2) Leave the area for 15 minutes to allow aerosols to settle. Replace contaminated PPE.
- 3) Upon return, mix spill with freshly made bleach to 10% final concentration.
- 4) Allow 30 minutes of contact time for disinfection.
- 5) Absorb spill with paper towels and dispose them into biohazard bags.
- 6) Use dustpan and broom to sweep up debris. Broken glass must be deposited into broken glass or sharps box.
- 7) Wipe the spill area clean using 10% bleach.
- 8) Dispose of contaminated PPE in autoclavable biohazard bags.

3B. In the event of injury or exposure

- 1) **CLEANSE WOUND:** Wash all wounds immediately with antiseptic soap and a high volume of water for up to 15 minutes.
- 2) **CONTROL BLEEDING**
- 3) **ACCIDENTAL INGESTION:** Rinse mouth with water but do not swallow.
- 4) **SEEK IMMEDIATE MEDICAL FOLLOW-UP** (*do not wait 24 hrs*)

Employees *and* students go to:

- **Employee Health Services** (during business hours: Monday-Friday, 7:30 am -4 pm).
Address/Location: 57 Bee Street, Charleston SC 29425; Phone: (843) 792-2991

