



Working with Viruses

1. Purpose:

To provide step by step guidance on how to safely use lenti- and adenoviral particles.

2. Scope:

Applies to Principal Investigators (PIs) and authorized laboratory personnel that have been pre-approved by the permit holder and the Department (the CAO) to work with virus while following strict operational protocols.

3. Prerequisites:

You are an authorized user of DB440 and either possess a CL2 permit for DB440 which explicitly lists viral work, or are included in your PI's. You have previously discussed viral work with the permit holder and with the Department (the CAO) and have reviewed additional safety precautions. Risk considerations must have been reviewed with the permit holder and the CAO. Only replication-defective lentiviral particles (2nd or 3rd generation lentiviral vectors) can be used. Nevertheless, as lentiviral particles are derived from HIV and stably integrate into the genome of the infected organism, risk consideration and increased safety measures must be taken seriously. Adenovirus must be E1 and E3 deleted to render viral particles replication defective and to reduce risk upon exposure. If you aim to work with viruses other than lenti- and adenovirus, the SOP must be updated accordingly.

4. Responsibilities:

It is the responsibility of all faculty, staff and students to follow the procedures described in this SOP. Working with viral vectors can be a real risk to the user and bystanders.

5. Personal Protection Equipment (PPE):



6. Procedure:

Before work:

Biosafety cabinets, tissue culture incubators, and low temperature storage units must be clearly labeled when used for working with viruses. Hang clearly readable signs on the BSC and the incubator. If possible, usage of the same compartment should be avoided by other users to minimize the risk of accidental spillage and handling.

Processing of adenovirus purification through cesium chloride (CsCl) density gradient combined with ultracentrifugation will take place in LM439. Please inform all other users 2 days prior and hang signs on the outside door to discourage other users from entering during this time due to the high risk of splashing.

Additional safety precautions:

All safety precautions as listed under 24 for working in Biosafety Cabinets apply. Additionally, the following precautions must be taken:

1. PPE: A lab coat and a double layer of nitrile gloves will be worn while working with viral vectors. If there is a splash risk, eye protection (safety glasses/goggles or a face shield) will also be worn. If there is high risk of exposure to aerosols, a N95 mask should be worn. All procedures will be performed in a biosafety cabinet. Upon contamination, the outer layer of the nitrile gloves only will be removed within the biosafety cabinet.
2. Surface Decontamination: Equipment and surfaces will be decontaminated with 70% ethanol prior to avoid contamination. The inside of the biosafety cabinet will be cleaned with clydox (freshly prepared following manufacturer instructions), or similar viricidal, with 10 min contact after working with viral vectors. Clydox must be wiped out with sterile water, followed 70% ethanol (failure to do so, will corrode the surface of the BSC).
3. Liquid Waste: Generally, volumes of viral vector will be kept at < 10 ml to avoid splash risks and severe exposure. Viral particles will be stored in 1 ml aliquots. All waste, both liquid and solid, will be decontaminated inside the BSC prior to removal. A designated electronic serological pipettor with a 0.2 µm filter will be used to handle liquids with a volume of > 1 ml. A designated set of pipettes will be used with filter tips for volumes < 1ml. Liquid waste will be decontaminated by the following procedure: Liquid waste containers will be emptied before use of viral particles, filled with undiluted bleach to 10%, and medium will be directly with serological pipettes and ejected into bleach to inactivate particles. Liquid waste from mammalian tissue culture will be brought to a final concentration of ~1% sodium hypochlorite (1:10 dilution from 12.5 % sodium hypochlorite stock to ~1%, VWR) within the biosafety cabinet, left for 30 minutes and then disposed of down the drain. Vacuum aspiration may cause the aerosolization of biological materials which can contaminate both the vacuum line and pump and thus will not be used for viral work. Electronic serological pipettes will be dedicated to viral procedures work only, and the 0.2 um filter replaced as appropriate and change outs documented.

4. Solid Waste: Waste that was in direct contact with viral particles will be covered in sufficient 1% sodium hypochlorite (1:10 dilution from 12.5 % sodium hypochlorite stock to ~1%, VWR) to decontaminate the virus and then sealed in leakproof containers (50 ml tubes or screw top plastic bottles for tips, autoclavable plastic bags for dishes) inside the BSC. Pipettes will be flushed with 1% bleach to ensure deactivation of particles within the pipette and proper immersion in 1% bleach. All solid waste will be left in bleach for 30 minutes, then the bleach will be disposed of as described for liquid waste prior, and then disposed in yellow bags/lined buckets for disposal. Waste will only be brought outside after the surface of the container has been sprayed with ethanol and when sealed in an airtight, leakproof container. RG2 solid waste will be collected in yellow bags/lined buckets with the biohazard symbol (double bagged) and disposed as biohazard waste by the facility.

7. Sharp Waste: The use of glass pipettes will be avoided to prevent aerosols from infiltrating the pumps and to avoid handling broken glass.

8. Spills Outside the BSC: The SOP guides personnel to immediately leave the room and place a yellow tape preventing re-entry. Personnel will wait 20 min before coming back in to deal with the spill allowing aerosols to settle. A spill kit is located right outside DB440 and includes PPE. *In addition to the safety procedures outline at the beginning, a CAN-95 mask (available in the spill kit) should be worn to avoid infection through aerosol. CAN-95 masks must be worn by all personnel entering a room with viral particles spilled outside the BSC.*

9. Spills inside the centrifuge: Viral centrifugation is only allowed in buckets closed with aerosol-tight lids that are only opened within the BSC. Following spills within these buckets, buckets and lids must be opened inside the BSC and placed inside a container to soak for 30 minutes in 1% sodium hypochlorite (1:10 dilution from 12.5 % sodium hypochlorite stock to ~1%, VWR).