



Lentiviral Vectors: Standard Operating Procedures

Containment Level

- ♦ Biosafety Level 2+.
- ♦ Biosafety Level (BL) 2 practices are adopted and rigorously followed.
- ♦ Some BL3 practices are also adopted.

Approval

- ♦ Experiments using lentivirus require IBC approvals before initiation of experiments.

Facility

- ♦ The designated laboratory must have aerosol containment equipment such as a biosafety cabinet, and a centrifuge with a sealed rotor or safety cups. Restricted entry to the lab.

Signage

- ♦ The biohazard sign is placed on the lab door.
- ♦ Also, a sign with the following information is posted in these areas:

“Staff should be aware that there is potential for recombinant virus generation. Since such recombinants may have enhanced host range afforded by the VSV-g pseudotype, all experimental materials must be handled with great care. Sharps must be eliminated from experimental procedures; in particular, procedures involved in pelleting virus should be reviewed carefully.”

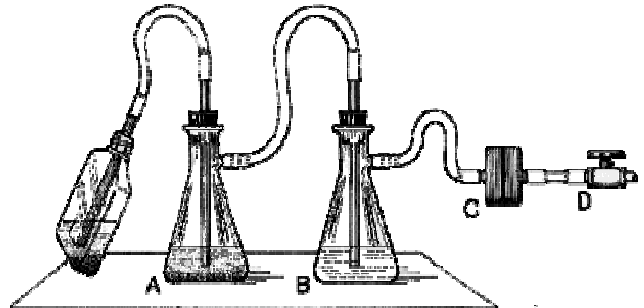
Personal Protective Equipment

- ♦ Gloves and lab coat

Work Practices

1. All procedures should be carried out according to the CDC and NIH guidelines for work under BL2 containment.

2. Keep laboratory door closed when experiments are in progress.
3. Use a biosafety cabinet for all manipulations.
4. Load ultracentrifuge buckets in biosafety cabinet; screw on cover, spray outside with alcohol.
5. For Aspiration- Use plastic vacuum flask (A) with a smaller vacuum flask (B) connected as a backup. Attach a HEPA capsule filter (C) to the small vacuum flask so nothing is sucked into the house vacuum (D). Attach these 3 items in series from the vacuum source in the hood.



6. Undiluted bleach should be pulled into the aspirator flask using suction to decontaminate tubing. Final concentration in the aspirator flask: 10% bleach.
7. The aspirator flask containing media, inactivated virus in the 10% final bleach solution can be drain disposed after 30 minutes.
8. Cells must be placed in a dedicated incubator, labeled “Lentivirus experiments only.”
9. No “sharps” (including needles and glass Pasteur pipets) may be used with these cultures or for harvesting virus pellets. Use plastic aspiration pipets instead of glass Pasteur pipets.



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10. Cells exposed to lentiviral vectors may not be removed from the designated room for experimental purposes unless inactivated by approved procedures.
11. Use plastic beakers containing 10% bleach inside the biosafety cabinet to soak and decontaminate all serological pipets and pipet tips before disposing in the biohazard box.
12. The double biohazard bag containing solid plastic waste must be autoclaved before disposal into regulated medical waste containers. Keep bag closed when it is being transported to the autoclave room.
13. Biosafety cabinet surfaces must be thoroughly wiped with effective disinfectant (70% ethanol/1% SDS) after the completion of each experiment.

Sources:

1. Donald E. Mosier, Ph.D., M.D., The Scripps Research Institute, "Safety Considerations for Retroviral Vectors: A Short Review"
2. Applied Biosafety, vol. 7, no.4, 2002
3. RWJMS Virology Lab

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