

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **Lentiviral Vectors**

<p>1. Health hazards</p>	<p>Lentiviral vectors are based on the Human Immunodeficiency Virus (HIV) which is the virus responsible for the development of Acquired Immunodeficiency Syndrome (AIDS). Lentiviruses are a subclass of retroviruses which are able to infect both proliferating & non-proliferating cells.</p> <p>The Lentiviral vectors have been modified to provide a safer version of the HIV virus. To increase the safety of lentivirus, some components of the HIV has been deleted and the components necessary for virus production are split across multiple plasmids. The third-generation lentiviral vectors encode only three of the nine HIV-1 proteins (Gag, Pol, Rev), which are expressed from separate plasmids to avoid recombination-mediated generation of a replication-competent virus.</p> <p>For further improves on the safety, the 3'LTR promoter was inactivated by a deletion that is inherited during the reverse transcription process in both the 5' and 3'LTRs, thereby "self-inactivating" both viral promoter elements.</p> <p>The major risks to be considered for research with HIV-1 based lentivirus vectors are: potential for generation of replication-competent lentivirus (RCL), and potential for oncogenesis.</p> <p>Lentivirus may be transmitted by:</p> <ul style="list-style-type: none"> • Penetration of the skin via puncture or absorption (though scratches, cuts, abrasions, dermatitis or other lesions) • Mucous membrane exposure of the eyes, nose, and mouth (through direct contact or aerosols)
<p>2. Designated Area</p>	<p>Work should be conducted in ABSL-2 facility, in a class II type A1 or A2 biological cabinet.</p>
<p>3. Training</p>	<p>Practical experience with microbiological and tissue culture technique and an understanding of this Standard Operating Procedures (SOP), is required.</p> <p>A training must be given by the Principal Investigator (PI) who supervise the experimental work.</p>
<p>4. Personal Protective Equipment (PPE)</p>	<p>Gloves (consider double-gloving for BSL-2+), Lab coat (dedicated lab coat for BSL2+) Eyes safety goggles, Closed Shoes.</p> <p>N-99 respirator mask covering the mouth and nose when there is a risk of aerosol/splash/spray of lentiviral particles outside the Biosafety Cabinet (BSC).</p> <p>Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.</p> <p><i>Personnel should not work with Lentivirus, if skin is cut or scratched.</i></p>
<p>5. Biosafety requirements and procedures</p>	<p>All work with lentiviral vectors must be performed in a BSL2 laboratory or BSL2+ laboratory if the insert is an oncogene or involves deletion/interruption in the process of cell cycle. The room should be suitable for tissue culture and equipped with a certified Class II Biosafety Cabinet (BSC).</p> <p>Access to the laboratory must be limited when the agent is in use.</p>

	<p>Vacuum lines to be used for aspiration must be equipped with an in-line HEPA filter and a vacuum flask.</p> <p>When concentrating the viral vector in an ultracentrifuge: rotors must be equipped with features (e.g., sealing O-rings) to minimize the risk of aerosol generation.</p> <p>Low-speed swinging-bucket centrifuge buckets must be equipped with aerosol-tight safety covers.</p> <p>Microcentrifuges must have aerosol-tight rotors capable of being removed while sealed so that the rotor can be opened/closed in the BSC.</p> <p>Doors must always be kept closed to maintain the BSL2/2+ containment.</p> <p>Centrifugation: Centrifuge tubes should be prepared and sealed/loaded and unloaded in the rotor/buckets in the biosafety cabinet. This includes methods to ensure tubes are properly balanced (unless the balance tube contains no infectious material). At the end of the procedure, rotors and/or buckets must be decontaminated.</p> <p>Vortexing must be done in the BSC.</p> <p>When the viral vectors are to be transported (from one location to another) a secondary container is required. The secondary container must be leak proof, rigid container, with a tight -fitting lid and labeled biohazardous.</p> <p>Storage of lentiviral stocks must be in leak-proof secondary containers (i.e. freezer boxes) in a -80° freezer clearly marked with biohazard label.</p>
6.Spill Kit	<p>The lab must have a spill kit, or the components of such readily accessible in the event of a spill. This comprises: an easy-to-read outline of the spill response SOP; gloves, masks, goggles, clean lab gown or lab coat, paper towels to absorb contaminated liquids, disinfectant, tongs or forceps to pick up broken glass and a biohazard bag.</p>
7.Sharps	<p>Sharps should be avoided whenever possible. Plastic aspirating pipets should be substituted for glass Pasteur pipets. If needles are required, they must never be re-capped, and must be disposed of in a sharps waste container immediately after use. While working with sharps inside the BSC, the sharps container must be kept inside the BSC.</p>
7.Decontamination	<p>Decontaminate work areas with 0.1% bleach or virusolve for 30 minutes. Follow with water.</p>
8. Spill and Accident Procedures	<ol style="list-style-type: none"> 1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure. 2. Cover the spill with absorbent material. Starting at the edges and work towards the center. 3. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant. 4. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 20 minutes: viscous spills require 30 minutes. 5. Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves 6. Discard absorbent material in Chemical waste bags. 7. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 20 minutes longer, and wipe with towels. 8. Discard all cleanup materials (soaked with disinfectant) in Chemical bag, and any contaminated PPE (pay special attention to gloves and shoe covers) in a biohazard bag. Close and secure the bags. 9. Place bag in a second biohazard bag, secure and disinfect by autoclaving.

	<p><u>Exposure:</u></p> <ol style="list-style-type: none"> 1. In case of skin contact, wash the affected area with soap and water for at least 15 minutes. In case of accidental puncture or needle injury, wash the affected area with antiseptic soap and warm water, while pressing the area of injury to draw blood out' for 15 min. Disinfect with iodine solution. Consult with Employee Health Center. 2. For eye exposure, flush with water for at least 15 minutes. Consult with Employee Health Center. Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit. Consult with Employee Health Center.
<p>9. Waste Disposal</p>	<p><u>Liquid Waste</u></p> <p>In case of aspirating into a vacuum flask, the vacuum flask must contain 1/10 volume concentrated bleach. The vacuum flask must have a final concentration of at least 1% bleach, for a minimum time of 30 minutes prior to drain disposal. Liquid waste may also be collected in the hood in a simple 500 ml bottle (like a bottle used to store cell culture medium) that contains 50 ml concentrated bleach (1% final v/v). Allow a minimum of 30minute incubation before pouring down the drain with copious amount of water.</p> <p><u>Solid Waste:</u></p> <p>Solid waste including pipet tips and tubes will be collected in a biohazard bag inside the Biosafety Cabinet. The biohazard bag must be inside a leak-proof, rigid container with a Biohazard label. Another option is to fill a plastic container with some bleach and put the solids inside this container while carrying out the procedure. At the end of the work the pipets and tips will be carefully Shake to get the liquids/bleach out and outside will be sprayed with 70% Ethanol and deposited into a biohazardous waste container. The bleach can be dumped down the sink with copious amount of water and the solid waste can be dumped in the biohazardous waste container.</p> <p>Autoclave all waste (1 hour at 121°C/250 °F, 15psi of steam pressure).</p>
<p>I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Lentivirus, and agree to follow these procedures.</p>	
<p>Name:</p>	<p>Title:</p>
<p>Signature:</p>	<p>Date:</p>

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