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| unicrest09-OHS | **Safe Operating Procedure**  ***Recombinant adenovirus and lentivirus safety***  **Generic safety methods for working with recombinant adenovirus and lentivirus** | | | |
| **S.O.P NUMBER** | | | **RISK ASSESSMENT NUMBER** | **RISK ASSESSMENT** |
| **Safe Work MS# XXXX** | | | RA XXXX | **Medium** |
| **DATE CREATED** | | **Employees Involved in SOP Creation** | | **REVIEW DATE** |
| XXXX | | **XXXX** | | XXXX |
| **Personal Protective Equipment Required** | | | | |
| * **Gloves – to be worn throughout entire procedure** * **Cuffed, long sleeved rear fastening laboratory gown** * **Covered shoes** | | | | |
| **Hazards** | | | | |
| (List hazards present during the undertaking of this method)   * **Handling, storage, transport and disposal of GMOs** * **UV light** * **Electrocution by electrical equipment** * **Hot/cold surfaces** * **Use of sharp instruments** | | | | |
| **Before work Commences** | | | | |
| (List anything required before work commences for the safe undertaking of this method) (delete any not applicable)   * **Obtain training; in PC2 facility use, general handling, storage, transport and disposal of GMOs and particularly in handling, storage, transport and disposal of virus** * **Ensure that you are aware of the locations of the following:**   + **Spill Kit**   + **Decontamination solution (1% Virkon)**   + **Eye Wash**   + **Fire extinguisher/blanket**   + **Exits** * **Risk Assessment and MSDS (Material Safety Data Sheet)- Ensure that you have read and understood for all the substances being used** * **Equipment**   + **Obtain training before using any equipment**   + **Ensure that you have read and understood the Risk Assessment and Safe Operating Procedure**   + **Check for safety and electrical compliance** * **Hazardous waste - Prepare appropriate labelled containers to put all hazardous waste solutions, tips and plastic ware into. Ensure that you have read and understood SOP and RA for disposal of biohazardous waste** | | | | |

1. Source of some equipment
   1. Virkon available from United Biosciences (07) 3219 2964, Cat# UB988-30
   2. Autoclavable Biohazard waste bags from Adelab 82347955
2. Equipment/materials

Virus work must be carried out in a PC2 facility such as room 6D316.2. Incubation of cells producing virus should be restricted to 6D316.2 and aseptic work must only be done in the biohazard cabinet in room 6D316.2. Centrifugation of lentivirus samples must be in sealed containers and rotor bucket lids must be used in the Allegra X-12R bench-top centrifuge (Beckman-Coulter) in room 6D316.2.

Purified lentivirus is centrifuged in common service Beckman Ultracentrifuge in room 6D307, 6E132 or 6E403, Beckman SW32 Ti rotor, tube supports (358156, Beckman) & floating spacers (355536, Beckman), Beckman tube sealer apparatus.

Keep all work surfaces and areas un-cluttered and free of absorbent materials to make clean-up easier in the event of a spill

1. Method

**Training:**

Users of adenovirus and lentivirus MUST be trained in general laboratory safety, Biosafety and also in specific use of the recombinant lentivirus. All procedures are in place to avoid the risk of un-intentional release to the environment and for the safety of the operator and others in the work place.

**Usage:**

Level 6, Room 6D316.2 PC2 room has a Biohazard cabinet and a CO2 incubator for sterile culture of cells or tissues treated with virus. Both doors to 316.1 and 316.2 must be closed when undertaking work in this room. The room is to be kept locked. Please see Angela Binns or Jess Hall for your own access code and location of the key to the outer room.

In all facilities, cuffed long sleeve rear-fastening gowns and gloves should be worn, along with the generic Biosafety and PC2 requirements.

Procedures involving aerosols must be done within the biosafety cabinet (e.g. opening centrifuge buckets or rotor)

**Cleaning and Waste disposal:**

1% Virkon Solution – 1 tablet is dissolved in 500ml room temp tap water and mixed well. Make solution fresh each week or when the colour of the solution turns from pink to white (usually about a week)

Collect contaminated tips, tubes and other items for disposal in a biosafety bag inside the biosafety cabinet. Seal and remove bag when finished.

Wipe down pipettes, tip boxes etc with 70% EtOH before removing from biosafety cabinet.

Liquid waste – Add to 1L schott bottle followed by an equal volume of 1% virkon solution. When full, add 2 Virkon tablets (1 per 500ml) mix well and decontaminate overnight. Dispose decontaminated liquid in sink 6D 316.1 (but not the sink dedicated to hand washing 6D316.2)

Sharps – into a metal pipette canister and autoclaved, prior to disposal in sharps bins.

Other waste – double bag using biohazard waste bags, the waste should go into a yellow biological waste bin.

Biohazard cabinet – After you have finished your work, clean the work surface of the cabinet with 1% virkon solution, then 70%EtOH and turn on UV when the hood is closed.

General surfaces- Clean thoroughly with 1% virkon solution and EtOH as above.

Re-usable containers- Soak in 1% Virkon for at least 2 hours, rinse thoroughly then clean as required. Autoclave.

Spills – Mop up thoroughly with 1% virkon solution soaked absorb material and disposed of all contaminated items, including materials used to mop up, in biohazard bags and autoclave.

**Storage:**

Stocks of concentrated adenovirus and lentivirus and other samples containing virus are clearly labelled in sealed containers and stored in the locked -80°C level 5 southern end of FMC behind Anatomy museum. 5D 400.4

All samples must be labelled and records of all virus sample must be kept. Virus stocks are recorded. Storage of virus treated samples is also recorded in the biosafety folder.

**Transport:**

Virus samples must be labelled and double contained in un-breakable containers when transporting outside a PC2 facility for storage or use within another certified facility.

Supervisor approval

Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Signature\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_