|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| unicrest09-OHS | **Safe Work Method Statement**  **Ultracentrifugation of Lentivirus** | | | |
| **Safe Work MS NUMBER** | | | **RISK ASSESSMENT NUMBER** | **RISK ASSESSMENT** |
| **Safe Work MS# xxxx** | | | **RA# xxxx** | **Medium** |
| **DATE CREATED** | | **Employees Involved in Safe Work MS Creation** | | **REVIEW DATE** |
| xxxx | | xxxx | | xxxx |
| **Personal Protective Equipment Required** | | | | |
| **Gown, Eye protection, gloves, shoes** | | | | |
| **Hazards** | | | | |
| * **Electrical, sharps, manual handling, Chemical, Biological** | | | | |
| **Before work Commences** | | | | |
| * Users must be trained in lentiviral work, have received Flinders University Biosafety Training and a PC2 facility induction. * Users must have read RA and SWMS before work | | | | |

*Reagents:* ***serum freee*** *SILAC DMEM medium*  
*Materials/Equipment:*

*Beckman Quickseal conical bottom polypropylene ultracentrifuge tubes,*

*tube supports & floating spacers,*

*Beckman Ultracentrifuge,*

*Beckman SW32 Ti rotor,*

*0.5ml conical screw cap microtubes,*

*20ml syringe, 20G 1¼ Safety IV catheters (Johnson & Johnson from Stores),*

*Beckman tube sealer, scalpel blades,*

*0.45um low protein binding filters*

*Method:*

* Collect virus from day 4 cultures in T75cm2 flasks.
* Filter harvest virus (about 15ml) with a 0.45μm filter (Room 6D316.2) directly into a 30ml ultracentrifuge tube (maybe need a 20ml syringe with the plastic sleeve from a 20G catheter to get in the opening of the tube). Fill tubes completely to the base of the opening shaft and apply cap.
* Transfer to Room 5D 214 (PC2) in double containment in unbreakable containers. Use a separate balance tube filled with **PBS** and balance until they are within 0.1g of each other. Seal tubes using heat sealer by placing metal cap on tube opening and applying the element until the cap slides down to the base of the opening (top of the bell top). Apply heat sink for 5 seconds to remove heat from cap. Remove cap and repeat for second tube.
* Place tube supports into ultracentrifuge buckets, insert tubes in opposite buckets (e.g. 1 and 4, 2 and 5 or 3 and 6), then floating spacers on top of tubes, then bucket lids. Make sure the rotor lid number matches the bucket number. Take rotor and buckets up to a common service ultracentrifuge on level 6 (Room 6E132). Place the rotor into the ultracentrifuge and then put in **all** the buckets according to their number (rotor spaces are also numbered).
* Spin for 90 mins at 4º C, 20,000 rpm. Stay with centrifuge until it is up to speed.
* After centrifugation carefully remove buckets into their rack, and bring rotor and buckets back to 6D316.2. In the biosafety cabinet using scissors (SWMS xxxx) gently snip off the top of each tube and remove about 1/3rd of the supernatant with a syringe and catheter sleeve. Use the tube cutter made by BME (no exposed blade involved) to carefully cut the top of the tube off to create an opening where you can pour off supernatant, and carefully drain. Turn tubes upside down in a rack and allow to drain for 1-10 minutes. See **SWMS xxxx**
* Resuspend virus pellet in 200µl of SILAC DMEM medium (about 150 fold concentration). Be aware that residual liquid in the tube will mean your final volume is larger than the volume you add. Also you need to allow for rinsing the tubes for maximum recovery.
* Virus infected media and consumables will be disposed of as per Biosafety SOP xxxx. Ultracentrifuge buckets and rotor and tube cutting scissors and device will be decontaminated as per SOP xxxx.
* Example :    Final volume desired = 400 uL  
  To each centrifuge tube add 100 uL of medium.   
  i) Gently pipette up and down and then leave to resuspend several hours at 4 degrees.  
  ii) Gently pipette up and down several times and then transfer virus solution to an eppendorf on ice.

iii)Rinse the ultracentrifuge tube with another 100ul medium

* Aliquot virus stock into screw capped eppendorf tubes in useful volumes (eg 20-50ul). Store aliquots at -80°C (location xxxx, transported in double containment in unbreakable containers).