Lentivirus Concentration

Biohazard Concerns

Lentivirus is a modified HIV virus and although unable to replicate in a host, it must be handled with caution. When working with these viruses, work only in a BL2+ designated hood or a viral vector room. All handling, storage and disposal of biohazard waste must be in accordance with Institute rules and regulations, OSHA, EPA and MWA.

Materials:

1.) SW-28 rotor with buckets and caps (Kept in 4C Refrigerator in Centrifuge Room)

2.) SW-28 thin walled ultra clear centrifuge tubes (Beckman # 344058)

3.) 0.45um filters (Corning # 431220)

4.) 60mL syringes with luer lok cap (Fisher # 13-689-8)

5.) p24 HIV ELISA Assay Kit (Cell BioLabs # VPK-108-HIV)

6.) 1.5mL RNase Free Eppendorf Tubes (Fisher # NC9445663)

7.) Spectrophotometer

Procedure:

Before Starting:

UV irradiate SW-28 thin walled tubes, SW-28 rotor buckets and SW-28 bucket caps in the hood overnight.

1.) Filter the 40mL of collected viral supernatant through a 0.45um filter using a 60mL syringe into a fresh 50mL centrifuge tube to remove any 293T packaging cells.

2.) Place the sterile SW-28 thin walled centrifuge tubes into the SW-28 rotor buckets using sterile forceps. Do not force the SW-28 tubes down into the rotor buckets, let them drop down slowly by themselves.

3.) Add 37mL of filtered virus to the sterile SW-28 thin walled centrifuge tubes.

Note: Two plates of virus production yield 40mL. Add 37mL of filtered virus to the SW-28 centrifuge tubes. If necessary, bring the volume up to 37mL with DMEM media.

Note: It is extremely important that every tube have exactly 37mL. No more and no less.

4.) Carefully screw on the caps to the SW-28 rotor buckets, matching the proper caps to the proper tubes. The numbers on the tubes and caps should be aligned.

5.) Take the SW-28 capped buckets into the centrifuge room and carefully attach the buckets onto the SW-28 rotor. Be sure the buckets are **securely** fastened onto the rotor. If they are not securely fastened, they will detach from the rotor and cause the centrifuge to become unbalanced and at high speeds, this could be disastrous.

Note: SW-28 buckets are specific to certain SW-28 rotors. Be sure that the rotor and buckets numbers match.

Note: The tubes inside of the buckets are full. Keep the buckets upright at all times and take care not to tilt them.

6.) Place the rotor with attached buckets into the centrifuge, being careful to not tilt anything even slightly. Be sure the rotor is in proper position in the centrifuge. Spin at 16,600 RPM for 90 minutes at 4ºC.

7.) When the spin is complete, remove the buckets carefully from the rotor, and bring back into the virus room. Place the rotor back in the 4C frig.

8.) Unscrew the caps, and aspirate the first 10mL of media in the tubes (to avoid spilling).

9.) Using sterile forceps, remove the tubes from the rotor buckets and place into a tube rack.

10.) Carefully aspirate off the media by tilting the tube so that the media flows towards the top and gets aspirated.

Note: The pellet will be invisible, but will be stuck in the bottom and should not move with the supernatant as you tilt.

Note: When tilting to aspirate, be sure you never tilt back. Tilting back can cause the supernatant to splash back into the bottom of the tube and disrupt the pellet.

11.) Add 500ul of plain OptiMEM media (no serum, no antibiotic) to the pellet. Cover the entire tube holder rack with saran wrap and place in the cold room overnight.

12.) The next morning, resuspend the virus carefully by pipet. Pipet up and down about 20 times, being careful to avoid making too many bubbles.

13.) Combine all the tubes together and mix gently. Aliquot the pooled virus into 1.5mL microfuge tubes. Each tube should contain 200-500ul.

9.) Remove 20ul of resuspended, combined virus from one of the tubes for titering.

10.) Freeze the tubes of concentrated virus at -80C. Be sure to label with the proper shRNA.

11.) Titer the virus according to the Cell BioLabs manual using a p24 HIV ELISA kit– determine the TU/mL concentration and calculate the amount of virus to add to your target cells depending on number of cells to infect and the MOI.