

## **Virus Concentration and Purification**

1. For volumes between 200 mL and 3 liters use Spectrum Laboratories, Inc. 500 kDa MWCO hollow-fiber ultrafilter (Cat.# X2-500S-300-02N). For volumes between 3 liters and 10 liters use Spectrum Laboratories, Inc. 500 kDa MWCO ultrafilter (Cat.# 3244229-100-01P).
2. The filters require a recirculation loop in order to function properly. To create the loop attach a 36 inch (or best length) section of Masterflex-25 tubing (Cole-Parmer Cat.#: 06508-25) to the inlet side of the hollow-fiber filter. Attach a 36 inch (or best length) section of C-Flex tubing (Cole-Parmer Cat.#: 06424-67) to the effluent side of the hollow-fiber and secure both sections with nylon cable ties. Attach the two ends of the tubing attached to the inlet and outlet of the filter to a Reservoir Cap. (FiberCell Systems Cat.#: A1006 or equivalent). This Reservoir Cap is designed to attach to standard Nalge square media bottles. Using the Luer connectors supplied with the ultrafilters attach a 3-6 inch section of the C-Flex tubing to the two outlet ports on the hollow-fiber.
3. Connect the Reservoir cap to a clean Nalge square 1-liter media bottle and place the length of Masterflex-25 tubing in an appropriate peristaltic pump. Flush the filter with water for approximately 10-15 minutes with the two outlet ports on the ultrafilter clamped off. Replace the water in the bottle and restart the pump. Open the ports on the ultrafilter and allow water to flow through the ports for 5-10 minutes. Disconnect the Reservoir Cap from the bottle and drain the system. The system is now ready for use.
4. Transfer the virus stock to be concentrated/diafiltered to a clean, sterile bottle with a 38 mm finish. Attach the Reservoir Cap assembly and close the outlet ports.
5. Start the recirculation using the peristaltic pump and adjust the flow to between 100 mL and 1-liter per minute.

6. When the flow has stabilized open one of the outlet ports and collect the permeate fluid into a clean, sterile bottle.
7. Continue recirculation until the desired level of concentration has been achieved. If diafiltration is required proceed to Step 8. Otherwise, proceed to Step 9.
8. For diafiltration continue the recirculation until the volume of the virus stock has been reduced by a factor of 10-fold. Close the outlet port and stop the pump. Resuspend the concentrated virus to the original volume with 25 mM Hepes buffer with 300 mM NaCl and 1% w/v BSA pH 7.0 Restart the recirculation and open the outlet port on the filter. When the volume has been reduced by a factor of 10 repeat the step using the same buffer. When the volume has been reduced to the desired level close the outlet ports, stop the pump and drain the system.
9. Filter sterilize the concentrated virus through a standard 0.22 micron filter unit and store at 2-8° C protected from light.