

Concentrating Viruses with Centrifugal Ultrafiltration Devices

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April, 2011

Purpose: This protocol describes how to concentrate viruses in liquid samples using an Amicon or Nanosep centrifugal ultrafiltration device. We use Amicons to concentrate medium volumes of samples (10s to 100s of mls) down to a final volume of ~4 ml. We use Nanoseps to concentrate smaller volumes of sample (<10 ml) down to a final volume of ~30 μ l.

Materials:

- Amicon or Nanosep with 100 kDa (kilodalton) NMWCO (nominal molecular weight cut-off) filter (Amicon: Millipore cat# UFC910024; Nanosep: Pall cat# OD100C34)
**Note: 200 kDa is the equivalent of ~10 nm, so 100 kDa will retain all known viruses
- centrifuge capable of 1000 x g
- vortexer
- parafilm

Amicon Protocol:

- 1) Add your sample to the upper reservoir of the Amicon
- 2) Centrifuge at 1000 x g (do not exceed 1000 x g) at 4°C in a swinging-bucket rotor
- 3) Centrifuge time will depend on the volume of sample and the amount of material in the sample
 - use an initial centrifuge time of 5 minutes or less and adjust this time depending on the volume of sample that has gone through the filter
 - **Do not let the filter go dry
- 4) Continue centrifuging until the sample is reduced to the desired volume (generally ~1 ml)
 - keep in mind that you will need to recover the sample using an additional 3 ml of liquid
- 5) Remove the concentrated sample from the upper reservoir and place it in a tube of your choice
- 6) Remove the upper reservoir from the Amicon device
- 7) Place a square of parafilm over the *bottom* of the upper reservoir (Figure 1)

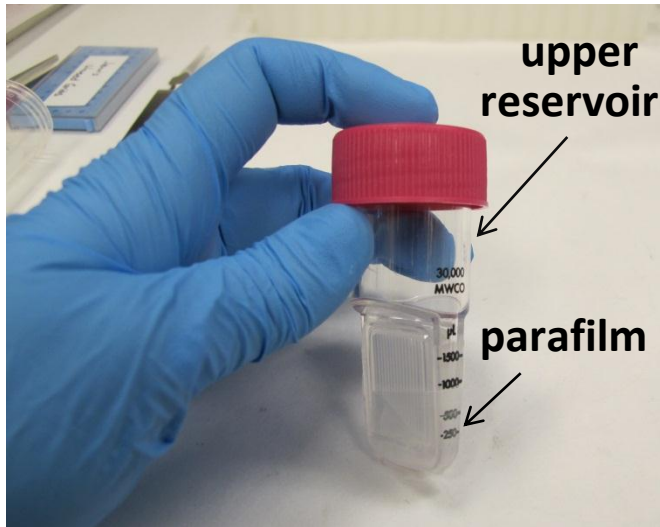


Figure 1. Placing parafilm over the bottom of the reservoir.

- 8) Add enough liquid to cover the filter (~1.5 ml)
 - you can recover the sample using whatever liquid you want (e.g. filtrate, buffer)
- 9) Vortex the upper reservoir (filter/parafilm side down) at a setting of 1200 for ~20 s (Figure 2)



Figure 2. Vortexing the upper reservoir.

- 10) Remove the liquid from the upper reservoir and add it to your recovered sample
- 11) Repeat steps 8-10 to fully recover your sample

Nanosep Protocol:

- 1) Add your sample to the upper reservoir of the Nanosep
- 2) Centrifuge at 1000 x *g* (do not exceed 1000 x *g*) at 4°C
- 3) Centrifuge time will depend on the volume of sample and the amount of material in the sample
 - use an initial centrifuge time of 5 minutes or less and adjust this time depending on the volume of sample that has gone through the filter
 - **Do not let the filter go dry
- 4) Continue centrifuging until the sample is reduced to the desired volume
- 5) Use a pipettor to remove the concentrated sample from the upper reservoir and place it in a tube of your choice
- 6) Add enough liquid to cover the filter (~10 µl)
 - you can recover the sample using whatever liquid you want (e.g. filtrate, buffer)
- 7) Swirl the liquid over the filter and stir it gently with a pipet tip (you can touch the membrane, but not so hard that you scrape chunks off of it)
- 8) Remove the liquid from the upper reservoir and add it to your recovered sample
- 9) Repeat steps 6-8 to fully recover your sample