

CHEMICAL PRECIPITATION METHOD FOR OBTAINING VIRUSES FROM SEAWATER

1. Prepare 20L seawater for chemical treatment. Pre-filter seawater through GF/D membrane to remove large particles. Re-filter the filtrate using a 0.22 μm Steripak filter. The cellular fraction will be retained on the filter, the virus fraction will be in the filtrate.
2. Treat the virus fraction (0.22 μm filtrate) with FeCl_3 to precipitate the viruses. Add 2ml of 10g/L Fe stock solution to each 20 L of filtrate. Shake vigorously for 1 min after addition of each 1ml FeCl_3 and repeat shaking several times. Let sit for 1 hr at RT.
3. Filter the FeCl_3 -treated filtrate using a 1.0 μm , 142mm, polycarbonate (PC) membrane filter on top of a 0.8 μm , 142mm, Supor support filter and attaching the filter apparatus to a peristaltic pump with a pressure gauge (maximum pressure = 15 psi). The FeCl_3 precipitate will be captured on the PC filter; the Supor is just for support in the 142mm stainless steel filtration apparatus. All of the 20L can be collected on a single 142mm PC membrane, but it is faster to change the PC membrane one or two times during the filtration.
4. Place all of the polycarbonate filters from the 20L into a 50cc centrifuge tube being careful not to scrape off any of the FeCl_3 on the edge of the tube (having precipitate facing out aids in dissolving the precipitate).
5. Add fresh 0.2M Ascorbate-0.1M EDTA-Mg buffer pH6-7 using about 1ml buffer for each 1mg Fe (this means about 20ml of 1x buffer for the 20L seawater precipitate or, alternatively, 10ml of a 2x concentrated buffer). Shake the tube vigorously. Incubate at 4°C, shaking when you can for the next day or so. You will see the solution change colors a number of times – that is okay. Leave this stored at 4°C until ready to analyze the viral fraction.

Reagents

10g/L Fe Stock Solution

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (FW=270.3) 4.83g
Q-water 100ml qs

Store at room temperature or 4°C.

0.2M Ascorbate-0.1M EDTA-0.2M Mg

EDTA-Mg₂ (FW=336.82) 3.37g (JT Baker L701)
Ascorbic acid (FW=176.12) 3.52g
5N NaOH 4ml
Q-water 100ml qs

Prepare fresh (w/in 48 hr of use). Dissolve EDTA-Mg₂ first in 90ml water. Once in solution, add ascorbic acid and NaOH. EDTA may precipitate out initially, but should go back into solution after addition of NaOH. pH should be between 6-7. QS to 100ml with water. Store dark at 4°C. This can be made as a 2x solution if less volume is desired to redissolve precipitate.

Notes:

1. The solution of ferric chloride hexahydrate is calculated based on the amount of iron, not on the amount of the salt. The stock solution has 10g Fe per liter. For precipitation, the final optimal concentration of **Fe** in seawater (SW) is 1mg Fe per liter SW which is equal to 2.9mg FeCl₃ per liter SW or to 4.83mg FeCl₃-6H₂O per liter SW.
2. The filters needed for this procedure are as follows:
 - Whatman glass fiber filter GF/D(cat.no. 1823-150; 2.7um retention) – 142mm diameter, but 150mm diameter will work with some trimming
 - Millipore Steripak GP10 or GP20 (cat.no. SPGPM10RJ or SPGPM20RJ; 0.22um) – the GP 10 works for <10L volumes and GP20 works for <20L volumes.
 - Pall Supor-800 filter (cat.no.60114; 0.8um) – 142mm diameter for large volumes.
 - GE Water Systems Polycarbonate membrane (cat.no.K10CP14220; 1.0um) – there is also a model number associated with this membrane (1216611). This is not easy to find but should be available for online ordering through Midland Scientific Inc.
3. If only working with a small volume of seawater (eg, 1 liter) can use 47mm filter membranes.
4. Filtration should be done at a maximum pressure of 15 psi. We used a peristaltic pump (~\$3000) to apply the pressure and a stainless steel Millipore filter rig (~\$2000) to hold our filters. For HugeCostSavings™©, we suggest finding an acrylic 142 mm filter holder (~\$400) and pressurizing your carboy with an air pump. Most lab vacuum pumps have an air-pressure outlet on the other side that can easily be adjusted to provide 15 psi pressure. Nalgene sells caps for their 20L carboys with fittings in the top that can be used for pressurization.