# Separation of free virus particles from sediments in aquatic systems

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## Abstract

This protocol is for the separation of free virus particles from sediments. It is from:

Danovaro, R., and M. Middelboe. 2010. Separation of free virus particles from sediments in aquatic systems, p. 74–81. In S. W. Wilhelm, M. G. Weinbauer, and C. A. Suttle [eds.], Manual of Aquatic Viral Ecology. ASLO.

Please see the full chapter for additional details.

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## Guidelines

The optimized protocol for the separation of free virus particles from sediments is shown in Figure 1.

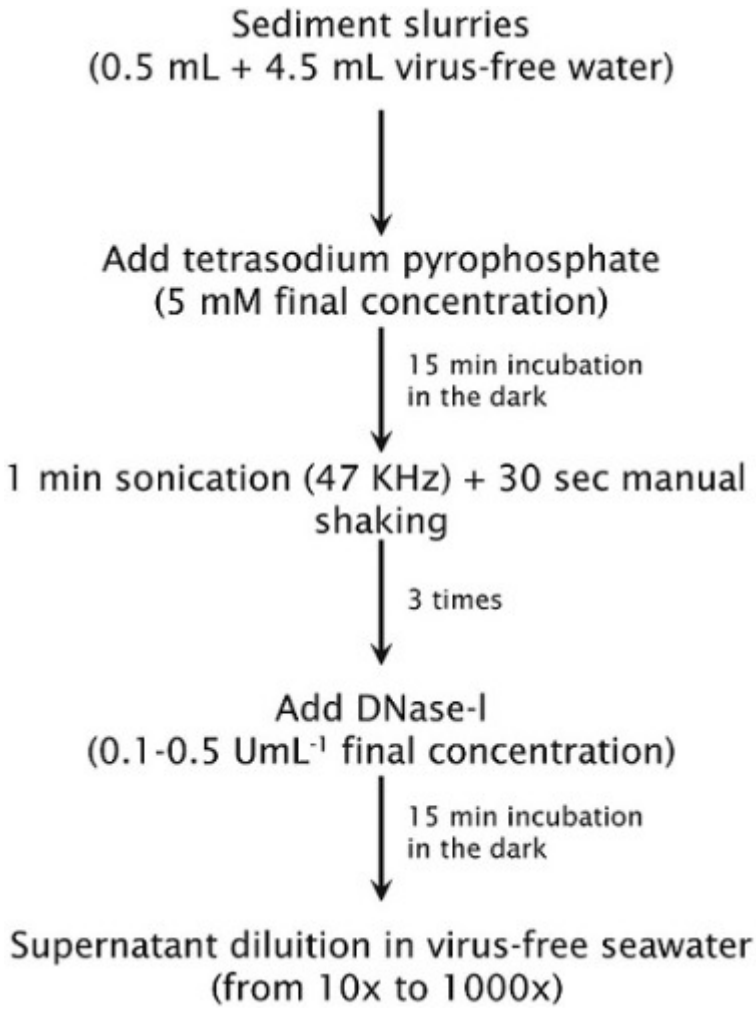


**Figure 1:** Protocol illustrating the steps required for the separation of viruses from the sediment particle and subsequent counting.

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## Protocol

**Step 1.**
Create sediment slurries by combining 0.5 mL sediment and 4.5 mL virus-free water.

**Step 2.**
Add tetrasodium pyrophosphate (5 mM final concentration).

**Step 3.**
Incubate 15 mins. in the dark.

DURATION
00:15:00

**Step 4.**
Sonicate at 47 KHz for 1 min. (1/3)

DURATION
00:01:00

**Step 5.**
Manually shake for 30 sec.

DURATION
00:00:30

**Step 6.**
Sonicate a second time at 47 KHz for 1 min.

DURATION
00:01:00

**Step 7.**
Manually shake a second time for 30 sec.

DURATION
00:00:30

**Step 8.**
Sonicate a third time at 47 KHz for 1 min.

DURATION
00:01:00

**Step 9.**
Manually shake a third time for 30 sec.

DURATION

00:00:30

**Step 10.**

Add DNase-I (0.1-0.5 $UmL^{-1}$ final concentration).

**Step 11.**

Incubate 15 mins. in the dark.

DURATION

00:15:00

**Step 12.**

Dilute supernatant in virus-free seawater (from 10x to 1000x).