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Viral enrichment from wastewater samples

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1 Works for me This protocol is published without a DOI.

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PROTOCOL CITATION

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38044

STEPS MATERIALS

NAME	CATALOG #	VENDOR
DNA/RNA Shield	R1100-50	Zymo Research

EQUIPMENT

NAME	CATALOG #	VENDOR
Ultracentrifuge	XPN-90	

SAFETY WARNINGS

Wastewater samples may contain potencially harmful pathogens, so always handle with gloves, work in a laminar flow cabinet, and pasteurize samples immediately after filtration. All equipment used up to this step must be washed in hypochlorite.

1 20m

Wastewater samples are centrifuged at **4500 rpm, 4°C 00:10:00** to remove debris

Volume processed depends on user preferences and equipment. For information on sampling, check our previous protocol : Sampling and viral concentration for SARS-CoV-2 detection in wastewater

2 1h

The supernatant is then filtered through a 0.45 µm membrane to remove eukaryotic and bacterial cells

Although the first step is optional, if your sample is very cloudy, it will take a lot of time for it to flow through

the filter. Also, if you see the flow stops, your membrane might be clogged, and you should replace it.

2.1 The flowthrough is pasteurized to inactivate pathogens at **60 °C** for **01:30:00** 1h 30m

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3 ⚠ 15m

25 mL of the flowthrough are then transferred to a 26,9 mL ultracentrifuge tube (compatible with Beckman Coulter Type 50.2 Ti Fixed-Angle Rotor) and spiked with **5.9 µl** of PP7 phage (this enacts as a protocol validation method later in the analysis).

Make sure your tubes are correctly balanced before you set up the Ultracentrifuge, according to manufacturer's instructions

3.1 Then, the result is ultracentrifuged at **100000 x g, 4°C 02:30:00** 2h 30m

Ultracentrifuge

Beckman Coulter XPN-90

Your ultracentrifuge must reach 100,000 x g and cool down to 4° C

4 The pellet is then resuspended in **200 µl** of Zymo DNA/RNA Shield, and stored at **-80 °C** or used for DNA/RNA extraction 25m

DNA/RNA Shield

by Zymo Research

Catalog #: R1100-50