



Jun 21, 2020

Viral enrichment from wastewater samples

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1 Works for me

This protocol is published without a DOI.



Institut Pasteur Montevideo

PROTOCOL CITATION

Iraola Lab 2020. Viral enrichment from wastewater samples . **protocols.io** https://protocols.io/view/viral-enrichment-from-wastewater-samples-bhd4j28w

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CREATED

Jun 10, 2020

LAST MODIFIED

Jun 21, 2020

PROTOCOL INTEGER ID

38044

NAME

STEPS MATERIALS

NAME	CATALOG #	VENDOR	
DNA/RNA Shield	R1100-50	Zymo Research	
EQUIPMENT			
NAME	CATALOG #	VENDOR	
Ultracentrifuge	XPN-90		

CATALOG #

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

VENDOR

Wastewater samples are centrifuged at \$\&\text{300 rpm, 4°C 00:10:00}\$ to remove debris



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

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

1h



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



06/21/2020

2.1 The flowthrough is pasteurized to inactivate pathogens at § 60 °C for © 01:30:00

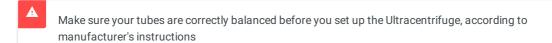
1h 30m



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3 **↑**

 \blacksquare 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 \blacksquare 5.9 μ l of PP7 phage (this enacts as a protocol validation method later in the analysis).



3.1 Then, the result is ultracentrifuged at \$\infty\$100000 x g, 4°C 02:30:00

2h 30m



The pellet is then resuspended in □200 μl of Zymo DNA/RNA Shield, and stored at 8-80 °C or used for DNA/RNA extraction

