Dec 02, 2021

Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation

Jacquelina.Woods [1], rachel.rodriguez [1]

[1]FDA

Jacquelina.Woods : Method Developer

dx.doi.org/10.17504/protocols.io.bx9ipr4e

GenomeTrakr
Tech. support email: **genomeTrakr@fda.hhs.gov**

Jessica Jones
US Food and Drug Administration

This method was developed at the FDA's Center for Food Safety and Applied Nutrition for GenomeTrakr's pandemic response project, monitoring SARS-CoV-2 variants in wastewater. Protocols developed for this project cover wastewater collection, concentration, RNA extraction, RT-qPCR detection, library prep, genome sequencing, quality control checks, and data submission to NCBI. This method describes the rapid concentration of intact viruses from wastewater using a combination of PEG precipitation and ultracentrifugation.

DOI

dx.doi.org/10.17504/protocols.io.bx9ipr4e

Jacquelina.Woods , rachel.rodriguez 2021. Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

GenomeTrakr, wastewater, SARS-CoV-2, virus, concentration, PEG precipitation, ultracentrifugation

protocol ,

Sep 15, 2021

Dec 02, 2021

Sep 15, 2021 Ruth Timme US Food and Drug Administration

Sep 23, 2021 Jessica Jones US Food and Drug Administration

53258

EQUIPMENT AND SUPPLIES:

1. Biological Safety Cabinet (BSC Type A2 or higher air exchange rate)
2. Nalgene PPCO 1L centrifuge bottles (Fisher Scientific, 05-562-25, or equivalent)
3. Adjustable Calibrated Micropipettor (1000 µl)
4. Hype-Wipe Disinfecting Towelettes (Fisher Scientific 14-412-56 or equivalent)
5. DNase/RNase-free microcentrifuge tubes 2.0 mL, non-stick, low retention, siliconized (Life Technologies AM12475)
6. Filter barrier aerosol resistant micropipettor tips DNase/RNase free (0.2 – 1000 µl)
7. Centrifuge capable of speeds of ≥12,000 x g (e.g., ThermoFisher RC6), and with rotors capable of holding 50ml conical tubes (ThermoFisher Scientific, 36-101-0816, or equivalent)
8. 50ml conical tubes (Fisher Scientific, 14-959-49A).
9. 100mL disposable graduated pipettes (Fisher Scientific, 03-395-177, or equivalent)
10. 50ml disposable graduated pipettes (Fisher Scientific, 13-678-11F, or equivalent)
11. FY14 50 carbon fiber rotor (ThermoFisher Scientific, 46922, or equivalent)
12. Ultracentrifuge capable of speeds of ≥170,000 x g (e.g., Sorvall WX90) and with rotors capable of holding 70ml ultracentrifuge tubes (ThermoFisher Scientific, 75000100, or equivalent)
13. Refrigerated incubator shaker (Eppendorf New Brunswick™ I24R shaker M1344-0014 or equivalent)
14. Refrigerated incubator shaker platform (Eppendorf, M1250-9902, or equivalent)
15. Refrigerated incubator shaker clamps with springs (Eppendorf, ACSB-500S, or equivalent)
16. Carbon Fiber Rotor F40L, 8 x 100 ml:(Thermo Scientific, 096-087057, or equivalent)
17. Carbon Fiber ROTOR SS F10-4X1000Y LEX (Thermo Scientific, 09-604-1053, or equivalent)
18. 70 mL Polycarbonate ultracentrifuge tubes w/aluminum cap tubes for F40L rotor (Fisher Scientific, 010-1333, or equivalent)
19. Vortex mixer (Labsource, S16-200, or equivalent)
20. Mettler Toledo™ NewClassic ME Precision Balance, 2200g (Fisher Scientific, 01-912-408, or equivalent)
21. Disposable transfer pipettes (Fisher Scientific, 13-711-22, or equivalent)

MEDIA AND REAGENTS:

☒ Glycine **Sigma**

1. **Aldrich Catalog #G7126** , or equivalent

☒ Guanidine Isothiocyanate **Thermo**

2. **Fisher Catalog #15535016** , or equivalent

☒ Hydrochloric Acid (HCl) **Fisher**

3. **Scientific Catalog #6000710** , or equivalent

☒ Nuclease Free Water **Life**

4. **Technologies Catalog #AM9937** , or equivalent

☒ Sodium Chloride (NaCl) **Sigma**

5. **Aldrich Catalog #S3014** , or equivalent

☒ Potassium Chloride **Sigma**

6. **Aldrich Catalog #P9541** , or equivalent

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

Potassium Phosphate dibasic (KH2PO4) **Sigma**

7. **Aldrich Catalog #P9791** , or equivalent

Sodium Phosphate dibasic anhydrous (Na2HPO4) **Sigma**

8. **Aldrich Catalog #S5011** , or equivalent

Tris (1 M) pH 8.0 RNase-free **Thermo Fisher**

9. **Scientific Catalog #AM9856** , or equivalent

EDTA (0.5 M) pH 8.0 RNase-free **Thermo Fisher**

10. **Scientific Catalog #AM9261** , or equivalent

10X Phosphate Buffered Saline **Sigma**

11. **Aldrich Catalog #P5493** , or equivalent

PEG 20000 **Sigma**

12. **Aldrich Catalog #81275-1KG** , or equivalent

Appropriate BSL-2 controls should be used when handling and processing wastewater samples. All steps for the concentration of viruses from wastewater should be completed in a BSC Type A2 or higher.

If sample is frozen, thaw under refrigeration (**2-8 °C**). For an 800 mL sample, thawing may take up to five (5) days.

To preserve RNA viruses, samples should be kept on ice whenever possible during the process. To avoid any RNA degradation, sterile glassware and DNase/RNase-free microcentrifuge tubes should be used.

Virus concentration 30m

1 Place **800 mL** of wastewater into an 1L centrifuge bottle.

2 Add **200 µL** of extraction control (concentration of 10^2 per mL), as described here: Preparation of Murine Norovirus for Use as an Extraction Control for Concentration of Viruses from Wastewater (protocols.io).

3 Add **40 mL** of 2X PEG.

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

2X PEG
by Jessica Jones,
US Food and Drug Administration

PREVIEW RUN

3.1 Dissolve components in ~ **700 mL** deionized or ultrapure water. Heating to **50 °C** is recommended to facilitate dissolution.

3.1.1 PEG-20000 **Sigma**
200 g **Aldrich Catalog #81275-1KG** , or equivalent

3.1.2 Sodium Chloride **Fisher**
34.8 g **Scientific Catalog #S271** , or equivalent.

3.2 Bring total volume up to **1 L** with deionized or ultrapure water.

3.3 Autoclave **121 °C** **00:15:00** . 15m

3.4 Store at **Room temperature** protected from light.

4 Add **400 µL** of 5N HCl.

5N HCl
by Jessica Jones,
US Food and Drug Administration

PREVIEW RUN

4.1 Measure **14.6 mL** of deionized or ultrapure water into sterile container.

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

4.2 Hydrochloric Acid (HCl) **Fisher Scientific Catalog #6000710**

Slowly add **10.4 mL** , or equivalent to the water.

This solution should be prepared under a chemical fume hood.

4.3 Store at **Room temperature** .

5 Shake briefly and place bottles in refrigerated shaker at **100 rpm, 6-8°C** for **02:00:00** . 2h

6 Centrifuge **8000 x g, 2-6°C, 00:30:00** . 30m

For ThermoFisher RC6, set acceleration at 7 and deceleration at 3, or equivalent speeds for other models.

7

Carefully pipette supernatant and discard, care should be taken not to disrupt the pellet.

Up to **15 mL** of residual supernatant may remain, it is not necessary to remove this volume and risk disrupting the pellet.

8 Resuspend pellet in **15 mL** of 0.05M glycine and transfer entire volume to a 50 ml conical tube.

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

0.05 M Glycine
by Jessica Jones,
US Food and Drug Administration

PREVIEW RUN

8.1 Glycine **Sigma** Mix **3.75 g** **Aldrich Catalog #G7126** in deionized or ultrapure water to **1 L**

8.2 Adjust pH **9.5**

8.3 Autoclave **121 °C** **00:15:00** 15m

8.4 Store at **2-8 °C**

9 Incubate **On ice** for **00:40:00 +/- 10 min** , vortex occasionally. 40m

10 Add **15 mL** of 2X tc PBS to neutralize. Shake by hand to mix.

Tissue Culture (tc) PBS
by Jessica Jones,
US Food and Drug Administration

PREVIEW RUN

10.1 Dissolve components in deionized or ultrapure water to 1L.

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

10.1.1 Sodium Chloride (NaCl) **Sigma**

8.0 g **Aldrich Catalog #S3014**

10.1.2 Potassium Chloride **Sigma**

0.2 g **Aldrich Catalog #P9541**

10.1.3 **0.12 g**

Potassium Phosphate dibasic (KH2PO4) **Sigma**

Aldrich Catalog #P9791

10.1.4 **0.91 g**

Sodium Phosphate dibasic anhydrous (Na2HPO4) **Sigma**

Aldrich Catalog #S5011

10.2 Adjust pH **7.5**

10.3 Autoclave **121 °C** **00:15:00** 15m

10.4 Store at **2-8 °C**

For 2X tc PBS, double components in 1.1 - 1.4

10.5

Alternatively, 1X and 2X PBS can be made by dilution of

10X PBS **Sigma**

Aldrich Catalog #P5493 into sterile deionized or ultrapure water.

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

11 Centrifuge at **3000 x g, 2-6°C, 00:15:00** . 15m

12 Transfer supernatant to clean ultracentrifuge tube (careful not to disturb pellet).

13 Bring total volume up to **65 mL** or **125 g** total weight (includes bottle and cap) with addition of 1X tc PBS.

Tissue Culture (tc) PBS
by Jessica Jones,
US Food and Drug Administration

PREVIEW RUN

13.1 Dissolve components in deionized or ultrapure water to 1L.

13.1.1 Sodium Chloride (NaCl) **Sigma**
8.0 g **Aldrich Catalog #S3014**

13.1.2 Potassium Chloride **Sigma**
0.2 g **Aldrich Catalog #P9541**

13.1.3 **0.12 g**
Potassium Phosphate dibasic (KH2PO4) **Sigma**
Aldrich Catalog #P9791

13.1.4 **0.91 g**

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

Sodium Phosphate dibasic anhydrous (Na2HPO4) **Sigma Aldrich Catalog #S5011**

13.2 Adjust pH **7.5**

13.3 Autoclave **121 °C** **00:15:00** 15m

13.4 Store at **2-8 °C**

For 2X tc PBS, double components in 1.1 - 1.4

13.5

Alternatively, 1X and 2X PBS can be made by dilution of 10X PBS **Sigma Aldrich Catalog #P5493** into sterile deionized or ultrapure water.

14 Balance tubes to within **0.05 g** of each other using 1X tc PBS.

15 Centrifuge at **170000 x g, 2-6°C, 00:45:00** . 45m

Minimum volume for ultracentrifugation using Fiberlite rotor and tubes is 50 mL.

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e

16 Discard supernatant and resuspend pellet in **800 µL** of 1X tc PBS.

17 Evenly distribute sample into four 2.0 ml microcentrifuge tubes.

18 Store concentrates from Step 17 at **-70 °C** or proceed directly to RNA Extraction from Wastewater Concentrates Using RNeasy and Zymo Kits (protocols.io).

Only 1 tube is required for RNA extraction. The remaining tubes should be stored at **-70 °C** as reserve.

Citation: Jacquelina.Woods , rachel.rodriguez Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation https://dx.doi.org/10.17504/protocols.io.bx9ipr4e