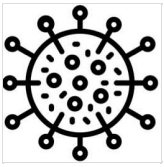

Apr 21, 2021

High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA

Aaron Topol[1], marlene.wolfe [2], Krista Wigginton[3], Bradley White[1], Alexandria B Boehm[2]

[1]Verily Life Sciences; [2]Stanford University; [3]Univ Michigan

1 *Works for me* dx.doi.org/10.17504/protocols.io.btyrnpv6

Wastewater-based epidemiology working group

Alexandria Boehm
Stanford University

ABSTRACT

This process instruction describes the steps for purification of nucleic acids from wastewater solids and preparation for downstream quantitative analysis with Reverse Transcriptase droplet digital Polymerase Chain Reaction (RT-ddPCR). Due to the large quantities of substances that have inhibitory effects on PCR in wastewater samples, a subsequent PCR inhibitor removal step is required after nucleic acid purification. Both steps of the process are carried out in a 96-well plate format.

This method uses the dewatered solids generated using this protocol: High Throughput pre-analytical processing of wastewater settled solids for SARS-CoV-2 RNA analyses.

RNA purification is carried out using a kit optimized for the purification of viral on for the Perkin Elmer Chemagic 360. Although only RNA is used in the downstream applications from this protocol, DNA is also eluted in this process. A crucial component of the purification kit are the magnetic particles coated with poly vinyl alcohol (M-PVA Magnetic Beads) which have a hydrophilic surface giving them an affinity for nucleic acids but not many other biological molecules. The workflow involves binding nucleic acids in a sample to the beads which are then transferred through a series of wash buffers to remove debris with a robotic head with magnetic rods.

The OneStep PCR Inhibitor Removal Kits are PCR inhibitor clean up kits that contain all the components needed for efficient removal of contaminants that can inhibit downstream enzymatic reactions (e.g. PCR and RT) from DNA and RNA preparations. The column matrices in these PCR inhibitor clean up kits have been specifically designed for the efficient removal of polyphenolic compounds, humic/fulvic acids, tannins, melanin, etc. from the most impure DNA and RNA preparations.

This process instruction applies to extraction of RNA from wastewater samples using the Chemagic™ Viral DNA/RNA 300 Kit H96 for the Perkin Elmer Chemagic 360 followed by PCR Inhibitor Removal with the Zymo OneStep-96 PCR Inhibitor Removal Kit.

DOI

dx.doi.org/10.17504/protocols.io.btyrnpv6

PROTOCOL CITATION

Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm 2021. High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.btyrnpv6

Citation: Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm (04/21/2021). High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. https://dx.doi.org/10.17504/protocols.io.btyrnpv6

CREATED

Apr 05, 2021

LAST MODIFIED

Apr 21, 2021

PROTOCOL INTEGER ID

48881

Citation: Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm (04/21/2021). High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. https://dx.doi.org/10.17504/protocols.io.btyrnpv6

MATERIALS TEXT

Equipment

- chemagic™ 360 instrument (Perkin Elmer, cat. # 2024-0020) equipped with a chemagic 96 Rod Head Set (cat. # CMG-370).
- Agilent Bravo Automated Liquid Handler
- Rainin Single-Channel Pipette L1000XLS+, L200XLS+, L20XLS+, L10XLS+, L2XLS+
- Rainin Multi-Channel Pipettes L12-1000XLS+, L12-200XLS+, L12-20XLS+, L12-10XLS+, L8-1000XLS+, L8-200XLS+, L8-20XLS+, L8-10XLS+
- Ice bucket or tray
- -80°C sample storage freezer
- Pipet Aid
- ALPAQUA Magnum EX Universal Magnet Plate (Cat. No. A000380)
- Beckman Coulter Allegra X-15R Centrifuge
- QInstruments BioShake XP

Reagents

- Chemagic™ Viral DNA/RNA 300 Kit H96 (Perkin Elmer, cat # CMG-1033-S)
  Lysis Buffer
  Binding Buffer
  Wash Buffer 3
  Wash Buffer 4
  Elution Buffer
  Magnetic Beads
  Proteinase K
- Zymo OneStep-96 PCR Inhibitor Removal Kit
  Prep Solution
  Nuclease Free Water

Consumables

- Chemagic™ Viral DNA/RNA 300 Kit H96 (Perkin Elmer, cat # CMG-1033-S)
  Deep Well Plates
  Low Well Plates
  Rack with Disposable Tips
- Zymo OneStep PCR Inhibitor Removal Kits
  Elution Plate
  Silicon-A™-HRC Plate
- Eppendorf twin.tec PCR Plate 96 LoBind, skirted
- Bio-Rad Microseal 'B' plate seal
- Rainin Low Retention, pre-sterilized, filter LTS tips 20 µL, 200 µL, 1000 µL
- Rainin Wide Orifice, Low Retention, pre-sterilized, filter LTS tips 1000 µL
- Disposable Serological Pipets
- 50 mL conical tubes
- Agilent Bravo Tips: 96LT 250 µL Sterile, Filtered
- Kim Wipes
- Blue Absorbent Pads
- Lab Tape

Samples and Test Materials

- Homogenized wastewater solid slurry in DNA/RNA shield prepared as described in

- DNA/RNA shield stock solution with BCoV spike-in from pre-analytical processing.
- SARS-CoV-2 gRNA positive control (ATCC® VR-1986D™) diluted and aliquoted at a concentration of 50 copies per µL.

DISCLAIMER:

Citation: Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm (04/21/2021). High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. https://dx.doi.org/10.17504/protocols.io.btyrnpv6

Preparation

1 If necessary, dissolve lyophilized Proteinase K in **11 mL** of nuclease free water following manufacturer directions.

Chemagic Viral DNA/RNA 300 Kit H96 **Perkin Elmer Catalog #CMG-1033-S**

2 Label one 50 mL conical tube as Lysis buffer.

3 Label a deep-well-plate as “Sample Plate”.

4 If the Chemagic 360 has not been used within 24 hours, run the protocol “Check Manifolds All.che” to prime the buffer manifolds.

.che files are protocols for the Chemagic 360 instrument and are available from Perkin Elmer.

Chemagic 360
Nucleic Acid Extractor
Perkin Elmer **2024-0020**

5 Elution Plates and Magbead Plates are generated in batches as needed with **60 µl** Elution Buffer and **150 µl** Magbeads per well. This can be done using a liquid handler such as the Agilent Bravo.

6 If there is condensation on the seal of the prepared Elution Plate, briefly spin the plate to collect the buffer in the bottom of the well.

Citation: Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm (04/21/2021). High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. https://dx.doi.org/10.17504/protocols.io.btyrnpv6

7 With the plate sealed, resuspend the magnetic beads in the prepared Magbead Plate by shaking in the BioShake XP at 1m **1300 rpm** for **00:01:00**

8 Check that the Viral RNA Buffers are connected to the Chemagic tubing. If not, perform a normal cleaning procedure, switch to Viral RNA Buffers and prime the system.

RNA Extraction with the Chemagic 360 Process Steps

9 Using a wide orifice 1000 µL pipette tip, transfer **300 µl** per well of homogenized sample (prepared as specified in High Throughput pre-analytical processing of wastewater settled solids for SARS-CoV-2 RNA analyses) to each well of the Sample Plate as follows:

9.1 Add **300 µl** of DNA/RNA shield stock solution with BCoV spike-in from pre-analytical processing to one well of the Sample Plate to serve as an extraction positive control.

9.2 Using a wide orifice tip, add the samples one at a time to ensure that a full **300 µl** is pulled up and no clumps prevent the aspiration of the sample.

10 Using normal pipette tips:

- Add **300 µl** of water to the indicated wells in the figure below to create extraction negative controls.
- Add **10 µl** of gRNA SARS-CoV-2 control (at a concentration of 50 cp/µl) to the indicated wells to represent the positive extraction control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1										Water	Water
B	Sample 2											
C	Sample 3											
D	Sample 4											
E	Sample 5											
F	Sample 6											
G	Sample 7											
H	Sample 8											Extraction Pos Control

Example set up of 96 well plate when 10 replicates are run of 8 samples. Water serves as negative extraction control in row 12. Row 11 is indicated to have water, but these wells are not used for downstream processing.

11 Mix the Lysis buffer according to the following volumes per sample/plate:

Citation: Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm (04/21/2021). High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. https://dx.doi.org/10.17504/protocols.io.btyrnpv6

A	B	C
Reagents	**Volume per Sample**	**Volume for Full Plate (with dead volume)**
Proteinase K	10 µL	1 mL
Lysis Buffer 1	300 µL	30 mL

Chemagic Viral DNA/RNA 300 Kit H96 **Perkin Elmer Catalog #CMG-1033-S**

12 Add **310 µl** of Lysis buffer solution mixed in step 11 to each well of the Sample Plate.

NOTE: The Proteinase K activity will decrease after incubation longer than **00:10:00** in Lysis Buffer. Ensure that all samples are mixed with Proteinase K / Lysis Buffer within the 10 min.

13 Set up the plate deck in the Chemagic 360 as follows:

Position 1: Rack with Disposable Tips
Position 2: Magbead plate, low-well-plate prefilled with 150 µL Magnetic Beads
Position 3: Sample Plate
Position 4: Empty Deep Well Plate
Position 5: Empty Deep Well Plate
Position 6: Empty Deep Well Plate
Position 7: Elution Plate, Deep Well Plate with 60 µL Elution Buffer
Position 8: Empty

Chemagic 360
Nucleic Acid Extractor
Perkin Elmer **2024-0020**

14 Select the protocol: “chemagic Viral300 360 H96 drying prefilling VD141210.che”

This .che file is available from Perkin Elmer

15 Make sure that all wells are selected and start the protocol.

When the protocol is complete, immediately proceed to PCR Inhibitor Removal or seal the Elution Plate and store on ice.

Citation: Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm (04/21/2021). High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. https://dx.doi.org/10.17504/protocols.io.btyrnpv6

16

Chemagic Clean Up

17 Dispose of empty Tip Rack (Position 1), empty Magbead plate (position 2) and Deep Well Plate with tips in it (Position 6) in a red biohazard waste bin.

18 Wrap Deep Well Plates in Positions 3, 4 and 5 in a blue absorbent pad, seal with lab tape, and dispose of as chemical waste.

PCR Inhibitor Removal with OneStep-96 PCR Inhibitor Removal Kit 13m

19 Prepare Silicon-A™-HRC Plate

The Silicon-A™-HRC Plate and Elution Plate come in the Zymo OneStep PCR Inhibitor Removal Kit

All the processes used to apply the Zymo OneStep PCR Inhibitor Removal Kit can be automated using a liquid handling robot. We use the Agilent Bravo to implement this section of the protocol.

19.1 Mount the Silicon-A™-HRC Plate onto an Elution Plate

19.2 Add **150 µl** Prep Solution to the wells on the Silicon-A™-HRC Plate by piercing the cover foil in the middle with the pipette tip.

19.3 Incubate for **00:05:00** 5m

19.4 Centrifuge the plate at exactly **3500 x g** for **00:05:00** . 5m

19.5 Discard Elution Plate with prep solution.

20 Add RNA extracts to prepped Silicon-A™-HRC Plate

20.1 While preparing the Silicon-A™-HRC Plate, allow the Chemagic Elution Plate (from step 16) to sit on the ALPAQUA Magnum EX Universal Magnet Plate for 2 minutes to pull down residual magnetic beads.

Citation: Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm (04/21/2021). High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. https://dx.doi.org/10.17504/protocols.io.btyrnpv6

Magnum EX Universal Magnet Plate
Magnet Plate
ALPAQUA A000380

20.2 Mount the prepped Silicon-A™-HRC Plate with the RNA extracts onto a new Elution Plate.

20.3 Transfer the full volume of extracted RNA in the Chemagic Elution Plate to the Silicon-A™-HRC Plate.

20.4 Centrifuge the plate at exactly **3500 x g** for **00:03:00** . 3m

20.5 Immediately seal the Elution Plate and store on ice until ddPCR analysis.

20.6 Discard the Silicon-A™-HRC Plate.

Citation: Aaron Topol, marlene.wolfe , Krista Wigginton, Bradley White, Alexandria B Boehm (04/21/2021). High Throughput RNA Extraction and PCR Inhibitor Removal of Settled Solids for Wastewater Surveillance of SARS-CoV-2 RNA. https://dx.doi.org/10.17504/protocols.io.btyrnpv6