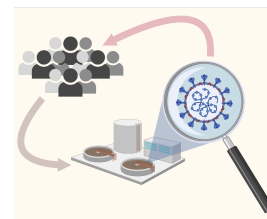


Jul 01, 2020

Version 2

Wastewater Concentration by Adsorption and Direct Extraction for SARS-CoV-2 RNA Detection and Quantification using RT-ddPCR V.2



DOI

<https://dx.doi.org/10.17504/protocols.io.bhiuj4ew>

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Coronavirus Method De...

2019-nCoV Wastewater ...



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Protocol status: Working

We use this protocol and it's working

Created: June 15, 2020

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Protocol Integer ID: 38196

Keywords: wastewater, SARS-CoV-2 RNA, ddPCR, wastewater for sar, processing wastewater, wastewater concentration by adsorption, direct extraction for sar, environmental specimens for sar, wastewater concentration, cdc interim laboratory biosafety guideline, pcr format, processing environmental specimen, droplet digital pcr, pcr, mce filtration, extraction, ddpcr, adsorption, sar,

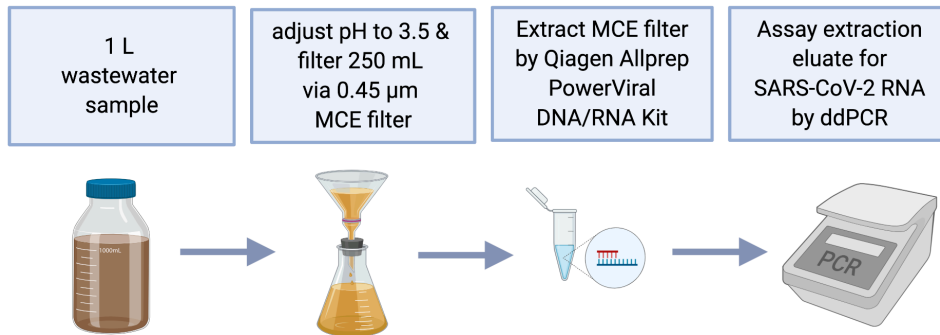
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Abstract

The following protocol describes the GERM Lab workflow for processing wastewater for SARS-CoV-2 via pH adjustment, MCE filtration, extraction, and assay by droplet digital PCR (ddPCR). It is synonymous to method A as described in Ahmed *et al.* 2020 (the referenced manuscript) with modifications to the consumables and PCR format. It is intended for use in a BSL2+ lab with extra precautions for processing environmental specimens for SARS-CoV-2 as described in the CDC Interim Laboratory Biosafety Guidelines.



Graphical abstract of the GERM Lab protocol for concentration SARS-CoV-2 RNA from wastewater and assaying by droplet digital PCR.

Guidelines

Please coordinate with your local biosafety committee to execute any wastewater handling and concentration procedures in accordance with biosafety standards for your institute and interim guidance issued by the CDC as referenced in the safety warning.

Materials

MATERIALS

☒ INFORCE 3 Intranasal Bovine Vaccine **Zoetis Catalog #INF-00089**

☒ Hydrochloric Acid **VWR International (Avantor) Catalog #87003-253**

☒ 2-Mercaptoethanol **MP Biomedicals Catalog #0219470580**

STEP MATERIALS

☒ INFORCE 3 Intranasal Bovine Vaccine **Zoetis Catalog #INF-00089**

☒ Hydrochloric Acid **VWR International (Avantor) Catalog #87003-253**

☒ 2-Mercaptoethanol **MP Biomedicals Catalog #0219470580**

☒ RT-ddPCR Oligonucleotides **Integrated DNA Technologies, Inc. (IDT) Catalog #Varies**

☒ 2019-nCoV RUO Kit **Integrated DNA Technologies, Inc. (IDT) Catalog #10006713**

☒ 2019-nCoV_N_Positive Control **Integrated DNA Technologies, Inc. (IDT) Catalog #10006625**

☒ One-Step RT-ddPCR Advanced Kit for Probes **Bio-Rad Laboratories Catalog #186-4021**

☒ Droplet Generation Oil for Probes **Bio-Rad Laboratories Catalog #1863005**

☒ ddPCR™ Droplet Reader Oil **Bio-Rad Laboratories Catalog #1863004**

In addition to the reagents listed above and the equipment and consumables listed within, this protocol requires access to typical microbiology equipment including pipettes and tips, microcentrifuge, an autoclave, a Class II biosafety cabinet, central vacuum line, and vortex.

Protocol materials

☒ Hydrochloric Acid **VWR International (Avantor) Catalog #87003-253**

☒ Droplet Generation Oil for Probes **Bio-Rad Laboratories Catalog #1863005**

☒ 2-Mercaptoethanol **MP Biomedicals Catalog #0219470580**

☒ INFORCE 3 Intranasal Bovine Vaccine **Zoetis Catalog #INF-00089**

☒ 2019-nCoV RUO Kit **Integrated DNA Technologies, Inc. (IDT) Catalog #10006713**

☒ One-Step RT-ddPCR Advanced Kit for Probes **Bio-Rad Laboratories Catalog #186-4021**

☒ ddPCR™ Droplet Reader Oil **Bio-Rad Laboratories Catalog #1863004**

☒ RT-ddPCR Oligonucleotides **Integrated DNA Technologies, Inc. (IDT) Catalog #Varies**

☒ 2019-nCoV_N_Positive Control **Integrated DNA Technologies, Inc. (IDT) Catalog #10006625**



Safety warnings

! Prior to processing wastewater samples for SARS-CoV-2, please review the CDC recommended biosafety guidelines for environmental specimen testing available at <https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html#environmental>. Work with your local biosafety committee to establish applicable biosafety protocols.

Before start

We are making this protocol available as a courtesy to our colleagues. We make no guarantees or warranties concerning its performance in other laboratories or settings.

Using this procedure at Notre Dame, we have successfully detected and quantified SARS-CoV-2 and pepper mild mottle virus in raw wastewater influent samples via the workflow described herein. Based on results described for murine hepatitis virus by Ahmed *et al.* 2020 (Method A: mean recovery 27%) adsorption direct extraction with pH adjustment will allow for reasonable recovery of SARS-CoV-2 RNA from wastewater. **Please note** that it is possible to perform this protocol without acidification (Ahmed *et al.* 2020 Method B mean recovery 61%), and with MgCl₂ amendment (Ahmed *et al.* 2020 Method C mean recovery 66%). **We have chosen to use acidification so that** we can compare our results in the current project with our historical sampling results.

This procedure relies on vacuum filtration for up to 4 hours to concentrate a 250 mL wastewater sample. It is advisable to use a central vacuum line for this task. However, it is also possible to filter a 100 mL sample in about 30 minutes.



Sample Collection

3h

- 1 Collect a  1 L sample of primary influent or raw wastewater (grab sample or composite) in a sterile sample collection bottle. Maintain the sample at  4 °C during transport to the lab. 2h
- 2 Upon sample receipt at the laboratory spike  1 mL of resuspended INFORCE 3 into the wastewater sample. INFORCE 3 contains bovine respiratory syncytial virus (BRSV) which serves as an RNA process control. BRSV is an enveloped single-stranded RNA virus. Spiking 1 mL of INFORCE 3 into 1 liter of wastewater yields approximately 10,000 BRSV gene copies per mL in the wastewater matrix. Mix thoroughly after spiking INFORCE 3 and allow the sample to incubate at  4 °C for  00:30:00 . Spiking the process control prior to any freezing and thawing allows for the effect of these processes on recovery to be examined. Other enveloped RNA process controls are also in use by other laboratories including murine hepatitis virus and bovine coronavirus.
 INFORCE 3 Intranasal Bovine Vaccine **Zoetis Catalog #INF-00089** 30m

- 3 If sample is to be concentrated immediately temporary storage at  4 °C is sufficient. If sample is to be processed at a future date (more than 2 days later), mix well, aliquot as suggested below, and store at  -80 °C until concentration is to be completed. Aliquot  500 mL of the wastewater sample for concentration as described below and store the remaining  500 mL at  -80 °C for any future analyses that may be required. 10m

HA Concentration

10h

- 4 Prior to concentration, sterilize the funnel top, funnel stem/membrane support, aluminum clamp, and filtration flask and GN-6 MCE filters by autoclaving. Alternatively, Millipore HAWP MCE filters can also be used in this protocol. 1h

Equipment

HAWP MF-Millipore Membrane Filter, 0.45 µm pore size

Membrane filter

Millipore

HAWP04700

https://www.sigmaaldrich.com/catalog/product/mm/hawp04700?lang=en®ion=US&gclid=CjwKCAjw8pH3BRAXEiwA1pvMsdoaQbbYstapLy8iGgQMUPUv1HEHZmOhSBoCPx8QAvD_BwE

0.45 um 47 mm

Equipment

GN-6 Metrice 0.45 um 47 mm MCE gridded

NAME

membrane filter

TYPE

Pall Corporation

BRAND

63020

SKU

<https://shop.pall.com/us/en/products/zid63020?CategoryName=ID34&CatalogID=Laboratory>

LINK

Equipment

Vacuum Filtration Assembly

NAME

Glassware

TYPE

Sigma-Aldrich

BRAND

Z290408

SKU

https://www.sigmaaldrich.com/catalog/product/sigma/z290408?lang=en®ion=US&cm_sp=Insite-_-caContent_prodMerch_raiOtherymICtr-_-prodMerch10-3

LINK

for 47 mm filters with glass support, NS 40/35 joints, 1 L flask (included)

SPECIFICATIONS



- 5 Assemble two vacuum filtration assemblies with GN-6 MCE filters for each sample that will be processed (two biological replicates). Handle filters and assemblies using proper aseptic technique. All concentration and processing should be carried out within a Class II Biosafety Cabinet. Attach each filtration flask to a central vacuum line using vacuum tubing. Include a bleach trap to avoid contaminating the vacuum line.

15m

****Note** vacuum times to filter the entire volume are up to 8 hours depending on wastewater sample turbidity, so a central vacuum line is strongly recommended.



Filtration columns and bleach traps set up within the BSC.

- 6 Using 2N Hydrochloric acid adjust the pH of each  500 mL wastewater sample to be concentrated to ~3 to 4. Add Hydrochloric acid in increments of 100 μ L to 10 μ L and measure the pH at each step using pH strips and sterile forceps. After acidification, gently mix the wastewater sample in a sealed vessel prior to beginning filtration. Please note that the protocol can also be performed with two variations: (1) no acidification natural pH or (2) amendment with MgCl_2 . Please see Ahmed *et al.* 2020 for more details on these variations.

15m



Equipment

pH Test Strips 0 - 14

NAME

pH test strips

TYPE

VWR International

BRAND

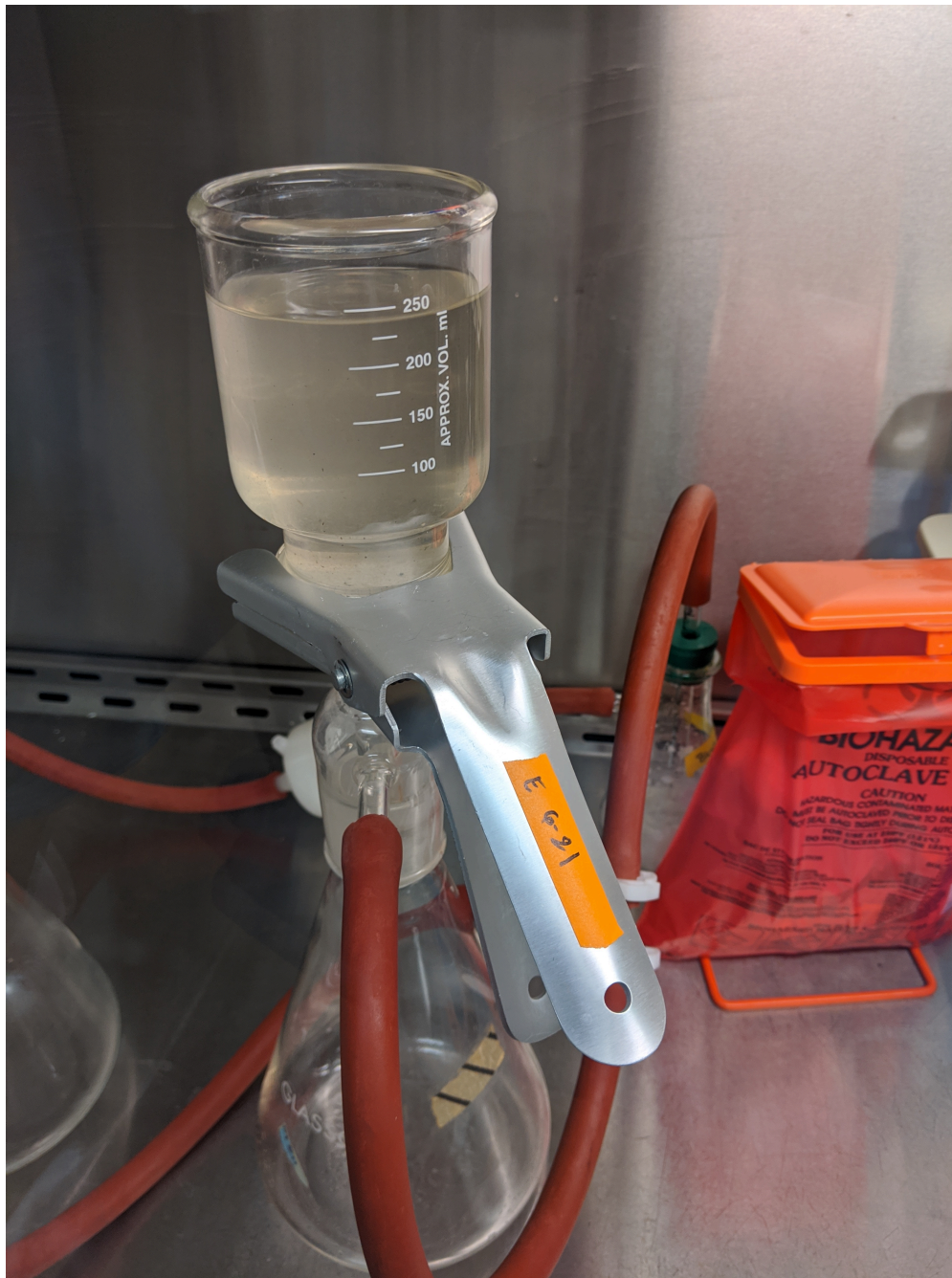
BDH35309.606

SKU

https://us.vwr.com/store/catalog/product.jsp?product_id=4645073^{LINK} Hydrochloric Acid **VWR International (Avantor) Catalog #87003-253**

- 7 Slowly add  250 mL of wastewater sample to a single filtration assembly funnel top in increments of approximately  50 mL . Allow the suspended settles to settle. Add the remaining  250 mL of wastewater sample to a second filtration assembly funnel top in the same manner. After wastewater sample has been added to each filtration assembly, slowly turn on the main vacuum line to begin filtration. Monitor for at least the first minute or two to ensure there is no leakage. Continue the vacuum until the entire  250 mL of each wastewater replicate has passed through the GN-6 MCE filter. Depending on the turbidity of the wastewater, filtration could take up to 8 hours.

8h



Raw influent sample at the beginning of filtration.



Filter with solids caked on after filtration.

- 8 After filtration, use a pair of sterilized forceps and aseptic technique to fold the GN-6 MCE filter and slide it into a 2mL PowerBead Tube, Garnet. If the filter is dry, use sterile water to rehydrate the filter prior to folding to prevent breakage.

15m

Equipment	
PowerBead Tube, Garnet	NAME
bead beating tube	TYPE
Qiagen	BRAND
13123-50	SKU
https://www.qiagen.com/us/products/discovery-and-translational-research/lab-essentials/plastics/powerbead-tubes/?clear=true#orderinginformation	LINK
PowerBead Tubes, Garnet 0.70 mm	SPECIFICATIONS

9 Store filters in PowerBead tubes at

🌡️ -80 °C

 until extraction.

RNA Extraction

3h

10 RNA Extraction is performed using the Qiagen Allprep PowerViral DNA/RNA Kit with modifications.



Equipment

Allprep PowerViral DNA/RNA Kit

NAME

DNA/RNA extraction kit

TYPE

Qiagen

BRAND

28000-50

SKU

<https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/allprep-powerviral-dna-rna-kit/?clear=true#orderinginformation>

LINK

AllPrep PowerViral DNA/RNA Kit (50)

SPECIFICATIONS

11

Sample Homogenization: Briefly centrifuge each PowerBead tube ~ $5000 \times g$ then add $650 \mu\text{L}$ of Warm Solution PM1 and $6.5 \mu\text{L}$ Beta-mercaptoethanol (Beta-ME) to each PowerBead tube. Homogenize the samples with 4 cycles for 20 seconds at 4.0 M/s on an MP Bio FastPrep 24. In between each 20 second homogenization cycle, briefly centrifuge the PowerBead tubes ~ $5000 \times g$. After the fourth and final homogenization cycle, centrifuge the PowerBead tubes at $13000 \times g$, 00:01:00 at room temperature. Transfer $450 \mu\text{L}$ of the resulting supernatant to a clean 2 mL Collection Tube.

2-Mercaptoethanol MP Biomedicals Catalog #0219470580

30m



Equipment

MP Bio FastPrep 24

NAME

bead beating grinder

TYPE

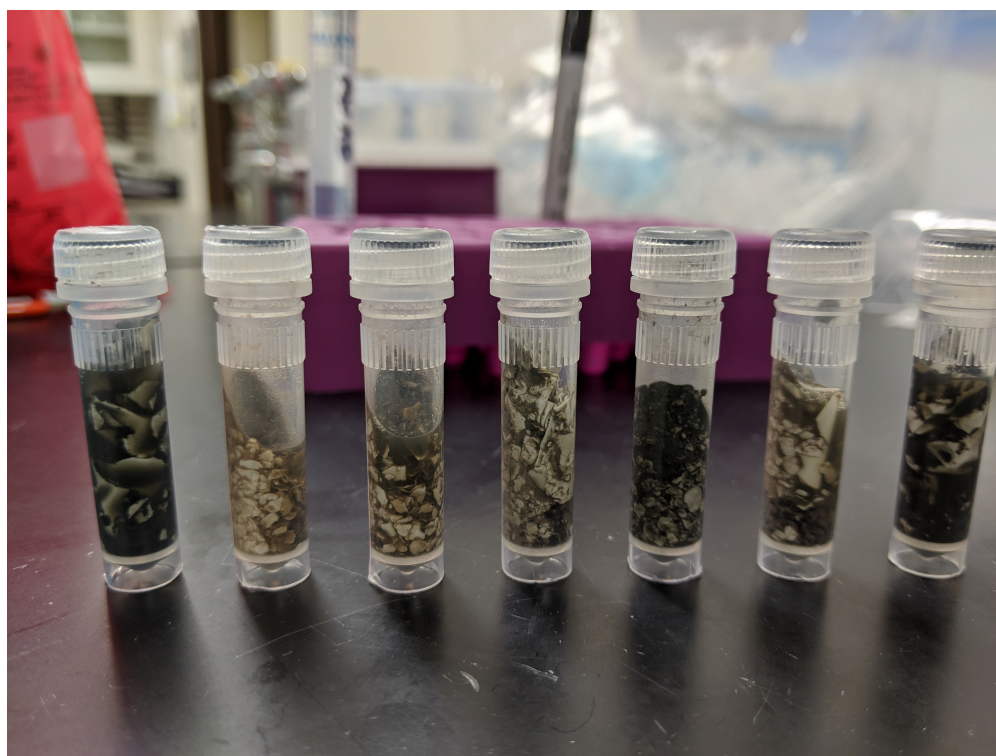
MP Bio

BRAND

116004500

SKU

<https://www.mpbio.com/us/fastprep-24-classic-instrument-1-each>^{LINK}



Homogenized filters after bead beating using an MP Bio FastPrep 24.



- 12 Add  150 μL of Solution IRS and vortex briefly. Incubate at  4 $^{\circ}\text{C}$ for  00:05:00 .
- 13 Centrifuge the 2 mL Collection Tube at  13000 x g, 00:01:00 . Transfer  500 μL of supernatant to a clean 2.2 mL Collection Tube avoiding the pelleted material.
- 14 Add  600 μL of Solution PM3 and  600 μL of Solution PM4 to each 2.2 mL Collection Tube and vortex briefly to mix.
- 15 Load  625 μL of mixture onto an MB Spin Column and centrifuge at  13000 x g, 00:01:00 . Discard the flow through and repeat until all the supernatant has been filtered through the Spin Column.
- 16 Shake to mix Solution PM5 and add  600 μL to the MB Spin Column. Centrifuge at  13000 x g, 00:01:00 .
- 17 Discard the flow through and add  600 μL of Solution PM4. Centrifuge at  13000 x g, 00:01:00 . Discard the flow through and centrifuge at  13000 x g, 00:02:00 .
- 18 Place the MB Spin Column into a clean 2 mL Collection Tube. Add  80 μL of RNase-free water to the center of the white column membrane and incubate for at least  00:01:00 . Centrifuge at  13000 x g, 00:01:00 and discard the MB Spin Column.
- 19 Spin the 2 mL Collection Tube at  13000 x g, 00:02:00 and transfer  60 μL of supernatant to a 1.5 mL Eppendorf LoBind Tube.





Equipment

DNA LoBind Tube 1.5 mL

NAME

Microcentrifuge tube

TYPE

Eppendorf

BRAND

022431021

SKU

<https://online-shop.eppendorf.us/US-en/Laboratory-Consumables-44512/Tubes-44515/DNA-LoBind-Tubes-PF-56252.html>

LINK

- 20 The DNA/RNA is now ready for downstream analysis. Store at  -80 °C until further analysis.

RT-ddPCR Oligos

- 21 The RT-ddPCR is performed using the One-Step RT-ddPCR Advanced Kit for Probes from BioRad. The concise steps below are taken from the package insert with a few modifications. For full details, please reference the BioRad Droplet Digital PCR Applications Guide and the User Manuals for the referenced equipment.

Please note that the protocol excludes details regarding experimental design such as technical replicates, biological replicates, negative extraction controls, no-template controls etc. Users are referred to the digital MIQE Guidelines for recommendations concerning quality assurance and control.

Huggett JF, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, Hellemans J, Kubista M, Mueller RD, Nolan T, Pfaffl MW. The Digital MIQE Guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments. Clinical chemistry. 2013 Jun 1;59(6):892-902.

- 22 Primers, probes, and control materials:

Unless otherwise specified, all oligonucleotide materials are purchased from Integrated DNA Technologies (IDT, Corallville, IA).

RT-ddPCR Oligonucleotides **Integrated DNA Technologies, Inc.**
(IDT) Catalog #Varies

22.1 BRSV primers and probes:

Boxus M, Letellier C, Kerkhofs P. Real Time RT-PCR for the detection and quantitation of bovine respiratory syncytial virus. J Virol Methods. 2005 May;125(2):125-30.

The probe for the BRSV assay specified below is on HEX (Channel 2)

Target region: beta-actin

Amplicon length: 124 bp

FWD Primer	5' GCA ATG CTG CAG GAC TAG GTA TAA T 3'
REV Primer	5' ACA CTG TAA TTG ATG ACC CCA TTC T 3'
Probe	/5HEX/AC CAA GAC T/ZEN/T GTA TGA TGC TGC CAA AGC A/3IABkFQ/

Table 1 - BRSV primers and probes for RT-ddPCR

22.2 SARS-CoV-2 primers, probes, and control plasmid:

The primer and probes set targeting the N1 and N2 genes are available premixed from IDT. The probes for both the N1 and N2 probes are on FAM (Channel 1).

2019-nCoV RUO Kit **Integrated DNA Technologies, Inc.** (IDT) Catalog #10006713

The N gene control plasmid is also available from IDT.

2019-nCoV_N_Positive Control **Integrated DNA Technologies, Inc.**
(IDT) Catalog #10006625

RT-ddPCR

7h

23 The RT-ddPCR is performed using the One-Step RT-ddPCR Advanced Kit for Probes from BioRad.

The kit referenced below is for 200 reactions and contains:

(1) Supermix: 500 µL x 2

(2) Reverse Transcriptase, RT:  200 μL x 2

(3) Dithiothreitol, DTT solution:  1 mL  300 millimolar (mM) x 2

 One-Step RT-ddPCR Advanced Kit for Probes **Bio-Rad**
Laboratories Catalog #186-4021

All components on the One-Step RT-ddPCR Advanced Kit for Probes are stable for 12 months when stored at  $-20\text{ }^{\circ}\text{C}$. Repeated freezing and thawing of the supermix is not recommended. DTT should be aliquoted to multiple tubes and stored at  $-20\text{ }^{\circ}\text{C}$ to minimize freezing and thawing.

24 Thaw all components on ice for  00:30:00. Mix each component thoroughly by vortexing each tube several times for  00:00:30 to ensure homogeneity because a concentration gradient may form during  $-20\text{ }^{\circ}\text{C}$ storage. Centrifuge briefly to collect contents at the bottom of each tube. 

25 Prepare samples at the desired concentration before setting up the reaction mix. Store samples at  $4\text{ }^{\circ}\text{C}$ while preparing the reaction mix.

26 Prepare the reaction mix for the number of reactions needed as shown below. The reactions should be setup on ice before droplet generation to prevent a nonspecific reverse transcription reaction from occurring. Assemble all required components except the sample, dispense equal aliquots into each reaction tube or well, and add sample to each tube or well as the final step. 

BRSV reagent volumes per reaction:

Reagent	Stock Conc.	Reaction Conc.	Volume per reaction (μL)
Water	n/a	n/a	 5.59 μL
Supermix	4x	1x	 5.25 μL
RT	n/a	n/a	 2.1 μL
DT	 300 millimolar (mM)	 15 millimolar (mM)	 1.05 μL
Probe	 100 micromolar (μM)	 250 nanomolar (nM)	 0.055 μL
Fwd	 10 micromolar (μM)	 900 nanomolar (nM)	 1.98 μL
Rev	 10 micromolar (μM)	 900 nanomolar (nM)	 1.98 μL
WW RNA	Variable*	Variable*	 4 μL



Total Volume per reaction:

22 µL

N1 or N2 reagent volumes per reaction:

Reagent (µL)	Stock Conc.	Reaction Conc.	Volume per reaction
Water	n/a	n/a	6.45 µL
Supermix	4x	1x	5.25 µL
RT	n/a	n/a	2.1 µL
DT	[M] 300 millimolar (mM)	[M] 15 millimolar (mM)	1.05 µL
IDT PreMix	Probe @ 250 nM		3.15 µL
	Primers @ 1000 nM		
WW RNA	Variable*	Variable*	4 µL

Total Volume per reaction:

22 µL

*Suggested input quantities of total RNA are 100 fg - 100 ng per reaction

- 27 Mix thoroughly by vortexing the reaction tubes or, if preparing reactions in a 96-well plate, by pipette mixing 5 times prior to transferring the reaction mix to the droplet generation cartridge.
- 28 Once the reaction mixtures are ready, place a DG8 Cartridge into a DG8 Cartridge Holder. Next load 20 µL of each reaction mix into the sample well of a DG8 Cartridge for QX200/QX100 Droplet Generator followed by 70 µL of Droplet Generation Oil for Probes into the oil wells, according to the QX100 or QX200 Droplet Generator Instruction Manual. After loading the sample and droplet generation oil, seal the DG8 cartridge in the DG8 Cartridge Holder using a DG8 Gasket.

Equipment

DG8™ Cartridges for QX200™/QX100™ Droplet Generator	NAME
Droplet Generation Cartridges	TYPE
BioRad	BRAND
1864008	SKU
https://www.bio-rad.com/en-us/sku/1864008-dg8-cartridges-for-qx200-qx100-droplet-generator?ID=1864008	LINK

Equipment

DG8 Cartridge Holder	NAME
Cartridge Holder	TYPE
BioRad	BRAND
1863051	SKU
https://www.bio-rad.com/en-us/sku/1863051-dg8-cartridge-holder?ID=1863051	LINK

 Droplet Generation Oil for Probes **Bio-Rad Laboratories Catalog #1863005**



Equipment

DG8™ Gaskets for QX200™/QX100™ Droplet Generator

NAME

DG8 Gasket

TYPE

BioRad

BRAND

1863009

SKU

<https://www.bio-rad.com/en-us/sku/1863009-dg8-gaskets-for-qx200-qx100-droplet-generator?ID=1863009>

LINK

- 29 Load the DG8 Cartridge Holder onto the QX200 Droplet Generator and close the lid to begin droplet generation.

Equipment

QX200™ Droplet Generator

NAME

QX200™ Droplet Generator

TYPE

BioRad

BRAND

1864002

SKU

<https://www.bio-rad.com/en-us/sku/1864002-qx200-droplet-generator?ID=1864002>

LINK

- 30 Once droplet generation is complete, carefully transfer  40 µL of the droplet oil emulsion from the DG8 Cartridge to a 96-well ddPCR plate.

**Equipment****ddPCR™ 96-Well Plates**

NAME

ddPCR™ 96-Well Plates

TYPE

BioRad

BRAND

12001925

SKU

<https://www.bio-rad.com/en-us/sku/12001925-ddpcr-96-well-plates?ID=12001925>

LINK

- 31 Once the droplets from all samples have been transferred to the 96-well ddPCR plate, seal the plate with plate heat seal foil using the PX1 PCR Plate Sealer.

Equipment**PCR Plate Heat Seal, foil, pierceable**

NAME

Heat Seal foil

TYPE

BioRad

BRAND

1814040

SKU

<https://www.bio-rad.com/en-us/sku/1814040-pcr-plate-heat-seal-foil-pierceable?ID=1814040>

LINK

Equipment

PX1 PCR Plate Sealer

NAME

Plate Sealer

TYPE

BioRad

BRAND

1814000

SKU

<https://www.bio-rad.com/en-us/sku/1814000-px1-pcr-plate-sealer?ID=1814000>

LINK

32 After sealing the 96-well plate, incubate the plate at  4 °C for  00:15:00 .



33 Place the sealed 96-well plate on the C1000 Touch Thermal Cycler and perform thermal cycling as specified in Table 2 below.

Equipment

C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module

NAME

Thermal Cycler

TYPE

BioRad

BRAND

1851197

SKU

<https://www.bio-rad.com/en-us/sku/1851197-c1000-touch-thermal-cycler-with-96-ndash-deep-well-reaction-module?ID=1851197>

LINK

Cycling Step
Cycles

Temperature (C)

Time

Number of

Reverse Transcription	🌡️ 50 °C	🕒 01:00:00	1
Enzyme Activation	🌡️ 95 °C	🕒 00:10:00	1
Denaturation	🌡️ 95 °C	🕒 00:00:30	40x
Annealing/Extension	🌡️ 59 °C	🕒 00:01:00	above
Enzyme Deactivation	🌡️ 98 °C	🕒 00:10:00	1
Hold	🌡️ 4 °C	Infinite	1

34 When thermal cycling is complete, allow the 96-well plate to incubate at 🌡️ 4 °C for 🕒 00:20:00 .



35 Place the 96-well plate in the QX200 Droplet Reader and ensure there is adequate ddPCR Droplet Reader Oil in the reservoir.

Equipment

QX200™ Droplet Reader

NAME

QX200™ Droplet Reader

TYPE

BioRad

BRAND

1864003

SKU

<https://www.bio-rad.com/en-us/sku/1864003-qx200-droplet-reader?ID=1864003>

LINK

🧪 ddPCR™ Droplet Reader Oil **Bio-Rad Laboratories Catalog #1863004**

36 Open QuantaSoft Software to set up a new plate layout according to the experimental design. Refer to the QX100 or QX200 Droplet Reader and QuantaSoft Software Instruction Manual.

37 Under Setup, double click on a well in the plate layout to open the Well Editor dialog box.



- 38 Designate the sample name, experiment type (ABS), ddPCR Supermix for Probes (No dUTP) as the supermix type, target name(s), and target type(s): Ch1 for FAM and Ch2 for HEX or VIC.
- 39 Select Apply to load the wells and, when finished, select OK.
- 40 Once the plate layout is complete, select Run to begin the droplet reading process. Select the appropriate dye set used and run options when prompted.
- 41 After data acquisition (~80s per well), select samples in the well selector under Analyze. Examine the automatic thresholding applied to the 1-D or 2-D amplitude data and, if necessary, set thresholds or clusters manually.
- 42 The concentration reported is copies per uL of RNA in the final 1x ddPCR reaction.