Precision Biomonitoring Inc.

TripleLock™ SARS-CoV-2 RT-qPCR Test

Product Information and Instructions for Use v. 5.1

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For *in vitro* Diagnostic Use

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Precision Biomonitoring TripleLock SARS-CoV-2 Test Device Description

Intended Use

The Precision Biomonitoring TripleLockTM SARS-CoV-2 test is a qualitative RT-qPCR test for detection of novel human coronavirus from clinical-collected nasopharyngeal swabs stored in viral transport media from patients who meet COVID-19 clinical and/or epidemiological criteria for testing. This device is not intended for use at the point of care (near patient). This test requires the use of an RNA extraction kit (M1 RNA 2.0 prep kit) and qPCR thermocycler (Franklin three9 device), sold in Canada by Precision Biomonitoring and authorized by Health Canada under Interim Order Authorization Reference #313602. Results are for the identification of SARS-CoV-2 RNA, however the assay does not differentiate between SARS-CoV-2 and SARS-CoV-1. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swabs during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories are required to report all positive results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. **For laboratory use only.**

Clinical Background Information

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). The novel coronavirus (SARS-CoV-2) is a new strain which has not previously been identified in humans. The current outbreak of SARS-CoV-2 has infected millions of individuals globally. The pathogen has high transmission rates and is spread through aerosolized droplets from the respiratory tract of infected patients. SARS-CoV-2 has extremely high virulence and infectivity.

The clinical impact ranges from asymptomatic infections to life threatening illnesses. When present, signs of infection include respiratory symptoms such as cough, shortness of breath, difficulty breathing and fever as well as fatigue, rash, loss of taste or smell, and digestive distress. In more severe cases, pneumonia, kidney failure and death can occur.

Intended User

The TripleLockTM SARS-CoV-2 tests is intended for use by qualified laboratory personnel, who have received specific training on the use of this test from Precision Biomonitoring only, for the in vitro qualitative detection of RNA from the SARS-CoV-2 in nasopharyngeal swabs from patients with or without signs and symptoms of infection who are suspected of COVID-19. This device is not intended for use at the point of care (near patient).

Test Principle/Principles of Operation

The Precision Biomonitoring TripleLock™ SARS-CoV-2 test is a multiplex RT-qPCR test designed for use with thermostable reagents that can be shipped, stored and run at room temperature. This approach is typical for identification of viral infections. The device requires RNA extracted from viral transport media into which a nasopharyngeal swab has been collected and a qPCR thermocycler on which to run the test. The tests are sold by Precision Biomonitoring (Catalogue numbers W000003, W000007, and F00004). The RNA extracted can be run immediately. Each RNA extract is interrogated for three targets simultaneously: E Gene, UTR and RNaseP as a human positive control target. Two viral targets are included to provide redundancy and reduce the likelihood of false negatives. More information on controls and targets is listed below in Assay Components.

Product Configuration

Precision Biomonitoring offers 7 formats for the TripleLock SARS-CoV-2 Test. Product configurations and requirements are listed below for each. These include:

1. F000004: 96-well plate format for use on the CFX96
2. W000003: 96 individual tests for the Franklin thermocycler
3. F000003: tests and consumables for the M1 RNA 2.0 Sample Prep and the Franklin thermocycler
4. W000007: 96 individual tests for the Maverick thermocycler
5. F000010: tests and consumables for the QIAamp Viral RNA Mini Kit and the Maverick thermocycler
6. F000011: tests and consumables for the QIAamp Viral RNA Mini Kit and the Franklin thermocycler
7. F000012: tests and consumables for the Biomeme M1 RNA 2.0 Sample Prep and the Maverick thermocycler

Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay Consumables Required and Provided as part of Precision Biomonitoring: Part # F000003

Source	Component	Description
(Precision Biomonitoring: W000003)	Precision Biomonitoring Triplelock SARS-CoV-2 Assay	Pre-aliquoted PCR tubes. Each well contains a single bead with a lyophilized triplex reaction containing all reagents to run the RT-qPCR reaction.
Sigma: V5005-100EA	2 mL self-standing tube	Tubes for storing extracted RNA from samples during or after the run setup process.
Mandel: GF-F171303	20 µL pipette tips	Tips for 20 µL fixed volume pipette
Mandel: GF-F171503	200 µL pipette tips	Tips for 200 µL fixed volume pipette
Biomeme: 3000567	Biomeme M1 sample prep cartridge for RNA 2.0	RNA extraction kit

Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay Equipment and Software Required

Source	Component	Description
Biomeme: 1000003	Biomeme Franklin three9 real-time PCR thermocycler	Thermocycler for conducting RT-qPCR
Biomeme: 1000013	Android smartphone with Biomeme Go App	Controller for Biomeme Franklin thermocycler
Biomeme: 2000006	Biomeme cloud	PCR Data Management Software
Mandel: 7030202020	20 µL fixed volume pipette	For transfer of exact liquid volumes
Mandel: 7030202024	200 µL fixed volume pipette	For transfer of exact liquid volumes

Note: Precision Biomonitoring does not provide external controls for use with the Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay. Quality control requirements should be performed according to applicable regulations and the laboratory's standard quality control procedures. Microbix RED-S19-01 swab controls have been validated for use with this kit as a positive control and can be used per the manufacturer instructions. Nuclease-free water (e.g. IDT: 11-04-02-01) can be used as a negative control.

Precision Biomonitoring TripleLock SARS-CoV-2 Assay 96-Well Plate Format (F000004)

The Precision Biomonitoring TripleLock SARS-CoV-2 Assay 96-well plate format (F000004) consists of a pre-aliquoted PCR 96-well plate of reagents. Each well contains a single bead with a lyophilized triplex reaction containing all reagents to run the RT-qPCR reaction.

An RNA extraction and compatible real-time PCR thermocycler are required to run this test, as well as a 20 µL pipette and sterile filter tips for loading/mixing samples into the plate and a suitable optical plate seal. These are not provided by Precision Biomonitoring. This format has been validated on the CFX96 (BioRad) and with the M1 RNA 2.0 Sample Prep (Biomeme)

Note: Precision Biomonitoring does not provide external controls for use with the Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay. Quality control requirements should be performed according to applicable regulations and the laboratory's standard quality control procedures. Microbix RED-S19-01 swab controls have been validated for use with this kit as a positive control and can be used per the manufacturer instructions. Nuclease-free water (e.g. IDT: 11-04-02-01) can be used as a negative control.

QIAamp Viral RNA Mini Kit and Franklin Thermocycler Format (F000011)

Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay Consumables Required and Provided as part of Precision Biomonitoring: Part # F000011

Source	Component	Description
(Precision Biomonitoring: W000003)	Precision Biomonitoring Triplelock SARS-CoV-2 Assay	Pre-aliquoted PCR tubes. Each well contains a single bead with a lyophilized triplex reaction containing all reagents to run the RT-qPCR reaction.
Luna Nanotech: LM-K7009-C	1.5mL microcentrifuge tube	Tubes for storing extracted RNA from samples during or after the run setup process and for setting up samples for extraction.
Mandel: GF-F171403	100 µL pipette tips	Tips for 100 µL adjustable pipette
Mandel: GF-F171703	1000 µL pipette tips	Tips for 1 mL adjustable pipette
Qiagen: 52906	QIAamp Viral RNA Mini Kit (250)	RNA extraction kit

Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay Equipment and Software Required

Source	Component	Description
Biomeme: 1000003	Biomeme Franklin three9 real-time PCR thermocycler	Thermocycler for conducting RT-qPCR
Biomeme: 1000013	Android smartphone with Biomeme Go App	Controller for Biomeme Franklin thermocycler
Biomeme: 2000006	Biomeme cloud	PCR Data Management Software
Rose Scientific: WCF01351	MAXpin C-12MT Micro-centrifuge w faxed Angle Rotor	Centrifuge for RNA extractions
Fisher: HC13001GL	Reagent Alcohol, 95%,	Reagent for RNA extractions
Fisher: 02-540G	PYREX™ Low Form Griffin Beakers	To hold ethanol during RNA extractions
Fisher: 03-007-41	Fisherbrand™ Polypropylene Graduated Cylinders	To measure ethanol for addition to RNA extraction reagents
	0.1-1 mL variable volume pipette	For transfer of exact liquid volumes
Luna Nanotech: PS1000S	10 - 100 µL variable volume pipette	For transfer of exact liquid volumes
Fisher: 03-448-20	96-Well PCR Tube Rack	To hold PCR tubes during run setup
Fisher: 22313630	80 Well Micro Tube Rack	To hold 1.5 mL tubes during RNA extraction and run setup

Note: Precision Biomonitoring does not provide external controls for use with the Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay. Quality control requirements should be performed according to applicable regulations and the laboratory's standard quality control procedures. Microbix RED-S19-01 swab controls have been validated for use with this kit as a positive control and can be used per the manufacturer instructions. Nuclease-free water (e.g. IDT: 11-04-02-01) can be used as a negative control.

Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay Consumables Required and Provided as part of Precision Biomonitoring: Part # F000012

Source	Component	Description
(Precision Biomonitoring: W000007)	Precision Biomonitoring Triplelock SARS-CoV-2 Assay	Pre-aliquoted PCR tubes. Each well contains a single bead with a lyophilized triplex reaction containing all reagents to run the RT-qPCR reaction.
Sigma: V5005-100EA	2 mL self-standing tube	Tubes for storing extracted RNA from samples during or after the run setup process.
Mandel: GF-F171303	20 µL pipette tips	Tips for 20 µL fixed volume pipette
Mandel: GF-F171503	200 µL pipette tips	Tips for 200 µL fixed volume pipette
Biomeme: 3000567	Biomeme M1 sample prep cartridge for RNA 2.0	RNA extraction kit

Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay Equipment and Software Required

Source	Component	Description
Anitoa: MQ-4164	Maverick thermocycler and tablet controller	Thermocycler for conducting RT-qPCR
Mandel: 7030202020	20 µL fixed volume pipette	For transfer of exact liquid volumes
Mandel: 7030202024	200 µL fixed volume pipette	For transfer of exact liquid volumes

Note: Precision Biomonitoring does not provide external controls for use with the Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay. Quality control requirements should be performed according to applicable regulations and the laboratory's standard quality control procedures. Microbix RED-S19-01 swab controls have been validated for use with this kit as a positive control and can be used per the manufacturer instructions. Nuclease-free water (e.g. IDT: 11-04-02-01) can be used as a negative control.

QIAamp Viral RNA Mini Kit and MaverickTest Format (F000010)

Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay Consumables Required and Provided as part of Precision Biomonitoring: Part # F000010

Source	Component	Description
(Precision Biomonitoring: W000007)	Precision Biomonitoring Triplelock SARS-CoV-2 Assay	Pre-aliquoted PCR tubes. Each well contains a single bead with a lyophilized triplex reaction containing all reagents to run the RT-qPCR reaction.
Luna Nanotech: LM-K7009-C	1.5mL microcentrifuge tube	Tubes for storing extracted RNA from samples during or after the run setup process and for setting up samples for extraction.
Mandel: GF-F171403	100 µL pipette tips	Tips for 100 µL adjustable pipette
Mandel: GF-F171703	1000 µL pipette tips	Tips for 1 mL adjustable pipette
Qiagen: 52906	QIAamp Viral RNA Mini Kit (250)	RNA extraction kit

Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay Equipment and Software Required

Source	Component	Description
Anitoa: MQ-4164	Maverick thermocycler and tablet controller	Thermocycler for conducting RT-qPCR
Rose Scientific: WCF01351	MAXpin C-12MT Micro-centrifuge w faxed Angle Rotor	Centrifuge for RNA extractions
Fisher: HC13001GL	Reagent Alcohol, 95%,	Reagent for RNA extractions
Fisher: 02-540G	PYREX™ Low Form Griffin Beakers	To hold ethanol during RNA extractions
Fisher: 03-007-41	Fisherbrand™ Polypropylene Graduated Cylinders	To measure ethanol for addition to RNA extraction reagents
Luna Nanotech: PS100S	0.1-1 mL variable volume pipette	For transfer of exact liquid volumes
Luna Nanotech: PS1000S	10 - 100 µL variable volume pipette	For transfer of exact liquid volumes
Fisher: 03-448-20	96-Well PCR Tube Rack	To hold PCR tubes during run setup
Fisher: 22313630	80 Well Micro Tube Rack	To hold 1.5 mL tubes during RNA extraction and run setup

Note: Precision Biomonitoring does not provide external controls for use with the Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay. Quality control requirements should be performed according to applicable regulations and the laboratory's standard quality control procedures. Microbix RED-S19-01 swab controls have been validated for use with this kit as a positive control and can be used per the manufacturer instructions. Nuclease-free water (e.g. IDT: 11-04-02-01) can be used as a negative control.

Assay Components

This assay targets: E Gene and UTR regions of the SARS-CoV-2 genome and RNaseP as a positive internal control. The RNase P gene is a single-copy human gene that encodes the RNA moiety for the RNase P enzyme, used as an endogenous human target in this assay to confirm successful amplification of the sample in the absence of SARS-CoV-2 and that sufficient material was used for the RNA extraction.

Fluorophores and quenchers for each target used in this device:

Name	**Probe Fluorophores and Quenchers**
E Gene_P	5'- TexRd-XN-BHQ2-3’
UTR_P	5' -FAM-BHQ1-3’
RNase P_P	5'-ATTO647NN-3IAbRQSp-3’

Specifications

Key Performance Specifications for the Precision Biomonitoring TripleLock™ SARS-CoV-2 assay.

Results were generated using the Biomeme M1 prep kit, and the Franklin real-time PCR instrument unless otherwise noted.

Specification	Details	Performance
Cross reactivity	Common respiratory pathogens (bacterial and viral) were tested using the M1 prep kit and the TripleLock™ SARS-CoV-2 assay to determine any cross-reactivity that could cause a false positive for SARS-CoV-2	No cross-reactivity was observed with pathogens tested. There is a low likelihood of false positives from common respiratory pathogens.
Limit of detection M1 RNA 2.0 Prep Kit and Franklin Thermocycler or Maverick Thermocycler	LOD was measured with contrived samples created from positive cell suspensions spiked into negative clinical samples in UTM.	The final calculated LOD for the assay is 1 x 10^4 copies/200 µL of initial sample input (corresponding to 2.12 x 10^2 per reaction, assuming no loss during extraction).
Limit of detection QIAamp Viral RNA Mini Kit and Franklin Thermocycler or Maverick Thermocycler	LOD was measured with contrived samples created from positive cell suspensions spiked into negative clinical samples in UTM.	For this test format, the LOD is 4.67 x 10^2 copies per reaction, assuming no loss during extraction.
Clinical evaluation	Across all test formats, 86 positive and 81 negative clinical samples were tested with both the TripleLock™ SARS-CoV-2 assay, and the CDC EUA test.	100% concordance to the reference test in all positive and negative samples for all test formats.

Cross-Reactivity Data for the Precision Biomonitoring TripleLock™ SARS-CoV-2 assay.

Results were generated using the Biomeme M1 prep kit, and the Franklin real-time PCR instrument.

Clinically confirmed specimen	5'UTR Cq	E gene Cq	RNaseP Cq
Viruses			
Negative nasal swab in UTM	0	0	35.90
Parainfluenza 1	0	0	26.03
Parainfluenza 2	0	0	27.16

Parainfluenza 3	0	0	25.91
Parainfluenza 4	0	0	29.74
Human Coronavirus 229E	0	0	23.81
Human Coronavirus HKU1	0	0	27.73
Human Coronavirus NL63	0	0	27.27
Human Coronavirus OC43	0	0	27.69
SARS-CoV2 (Pos control)	30.58	27.00	25.49
Adenovirus	0.00	0	28.29
Human Metapneumovirus	0	0	28.32
Influenza A – H1N1	0	0	27.18
Influenza A – H3N2	0	0	26.20
Influenza B	0.00	0.00	29.76
Enterovirus (not subtyped)	0.00	0.00	27.57
Respiratory syncytial virus	0.00	0.00	27.12
Rhinovirus	0.00	0.00	30.14
Haemophilus influenzae	0.00	0	26.96
Streptococcus pneumoniae	0	0	27.13
Streptococcus pyogenes	0	0	27.83
Candida albicans	0	0	28.25
Pseudomonas aeruginosa	0.00	0	27.05
Staphylococcus epidermis	0	0	27.28
Staphylococcus salivarius	0.00	0	27.46
Mycoplasma pneumoniae	0	0	27.43
Chlamydia pneumoniae	0	0	27.08
Legionella pneumophila	0	0	24.20
SARS-CoV-1	16.38	13.26	0.00
MERS	0.00	0.00	29.20
Bordatella pertussis	0.00	0.00	39.59
Pneumocystis jiroveci*	0.00	0.00	n/a

*Control provided as extracted nucleic acid, therefore RNaseP could not be recorded.

Repeatability Data for the Precision Biomonitoring TripleLock™ SARS-CoV-2 assay.

Results were generated using the Biomeme M1 prep kit, and the Franklin real-time PCR instrument.

A. Within run variation. Mean and standard deviation of the Cq values are given for each instrument across all days.

	Franklin D13AB6A254B2			Franklin CC92BD2871DB			Franklin C43440902003		
	UTR	**E Gene**	**RNaseP**	**UTR**	**E Gene**	**RNase P**	**UTR**	**E Gene**	**RNase P**
Mean	28.98	26.88	27.28	29.06	26.95	27.39	29.03	26.96	27.76
SD	0.374797	0.336693	0.472211	0.588501	0.201179	0.478864	0.501677	0.442525	0.492146

B. Between run variation. Mean and standard deviation are given for Cq values across all days, instruments, and replicates.

	UTR	**E Gene**	**RNaseP**
Mean	29.02	26.93	27.48
SD	0.497045	0.343397	0.522158

C. Within day variation. Mean and standard deviation are given for Cq values for all values across all instruments generated on the same day.

Day		**UTR**	**E Gene**	**RNaseP**
1	Mean	28.91	26.84	28.91
1	SD	0.528507	0.528507	0.528507
2	Mean	28.67	28.67	28.67
2	SD	0.453346	0.453346	0.453346
3	Mean	29.07	29.07	29.07
3	SD	0.318276	0.318276	0.318276
4	Mean	29.28	29.28	29.28
4	SD	0.450843	0.450843	0.450843
5	Mean	29.19	29.19	29.19
5	SD	0.458395	0.458395	0.458395

D. Day to day variation. Mean and standard deviation are given for Cq values for all days, instruments and replicates.

	UTR	E Gene	RNaseP
Mean	29.02	26.93	27.48
SD	0.497045	0.343397	0.522158

E. Machine to machine variation. Mean and standard deviation are given for Cq values for each machine across all days and replicates.

	Franklin D13AB6A254B2	Franklin CC92BD2871DB	Franklin C43440902003
Mean	27.72	27.80	27.92
SD	0.996085	1.013425	0.979807

Specimen Collection

Specimen collection is not included as part of TripleLock™ SARS-CoV-2. It is intended for use with RNA extracted from viral transport media from a nasopharyngeal swab collection made by a health care professional. Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. Specimens can be stored for up to 72 hours at 2-8°C before RNA extraction.

Precision Biomonitoring TripleLock SARS-CoV-2 Test Device Operation

Contents: Lyophilized reagents in PCR tubes

Instructions for Use:

Each Precision Biomonitoring SARS-CoV-2 Test contains everything needed for testing a nasopharyngeal swab collected from a patient and stored in viral transfer media. Each format requires an RNA extraction and a thermocycler to run the test. Below are instructions for each of the two extraction and two thermocycler options provided by Precision Biomonitoring as accessories with the Precision Biomonitoring TripleLock SARS-CoV-2 test, as well as for the 96-well plate format with a third thermocycler.

Set up:

For all device formats, you will need:

- qPCR thermocycler and control app or software
- Lyophilized qPCR reagents for the PBI SARS-CoV-2 Test
- RNA extraction kit
- Gloves
- Eye protection
- Lab coat
- Any other appropriate PPE
- Appropriate waste disposal

Wear a lab coat, gloves and eye protection This device is not intended for use at the point of care (near patient). Wipe down the test surface with 10% bleach, then with distilled water before beginning testing.

RNA Extraction

M1 RNA 2.0 Prep Kits

Additional items required:

- 20 uL fixed volume pipette
- 200 uL fixed volume pipette
- 20 uL tips
- 200 uL tips
- M1 RNA 2.0 Sample Prep kits

The sample preparation is done with the M1 RNA 2.0 prep kit, which comes with a syringe, transfer tip to attach to syringe, and cartridge containing extraction buffers in compartments covered by foil. This prep kit is used to extract RNA from the viral transfer media into which a nasopharyngeal swab from a patient has already been collected. The SARS-CoV-2 virus is inactivated in the first buffer of this cartridge after incubation. The entire process takes approximately 3 minutes after initial incubation (see step 3 below).

Step 1: Set out (but do not yet open) the number of extraction kits equal to the number of samples to be processed in the run.

Step 2: Wearing gloves, lab coat and eye protection, open the foil package containing a single M1 RNA 2.0 prep kit for each of the samples you intend to test. Screw the syringe to the transfer tip. Using the tip, pierce 2 holes in foil of the first cartridge compartment.

Step 3: Using a disposable plastic transfer pipette, or fixed volume pipette and correct tips (see proper pipetting instructions below), transfer 200 µL of viral transfer media into the first compartment of the M1 prep kit. Repeat for all samples you will test. Allow to incubate for 10 minutes.

Step 4: Place the tip of the assembled syringe into the first compartment and slowly draw liquid up, and push back down, a for a total of 10 pumps. Move slowly through this step to prevent clogging of the filter or creation of bubbles in this buffer.

Step 5: At the last pump, push all the liquid out of the syringe. Pierce 2 holes in the foil of compartment 2. Move syringe only to this compartment, and pump liquid up and back down once.

Step 6: Repeat for compartment 3 and 4, pushing liquid completely out of syringe at each compartment switch. No liquid is carried over between steps.

Step 7: Compartment 5 contains absorbent material to dry the filter contained in the syringe tip. After expelling all liquid into compartment 4, pierce the foil on compartment 5. Insert the syringe tip into the absorbent material and pump up and down very rapidly at least 20 times. Visually check the tip and syringe for any remaining liquid, and repeat drying pumps as needed to ensure no liquid is transferred to the final compartment.

Step 8: One clear of all liquid, use the syringe tip to pierce the foil on the final compartment of the cartridge and pump up and down 5 times. The extracted RNA is now in the liquid in this final compartment. Transfer to a free-standing 2.0mL screw cap tube using the syringe. Repeat for each sample. Use this directly in the test strips as outlined below in RT-qPCR Setup for your test format.

QIAamp Viral RNA Mini Kit

Additional items required:

- 200 uL variable volume pipette
- 1000 uL variable volume pipette
- 100 uL tips
- 1000 uL tips
- QIAamp Viral RNA Mini Kit

The sample preparation is done with the QIAamp Viral RNA Mini Kit. The full handbook is provided, and can also be found at:
https://www.qiagen.com/us/resources/resourcedetail?id=c80685c0-4103-49ea-aa72-8989420e3018&lang=en

Before you start:

- If starting a new kit, refer to the full handbook to prepare the reagents. Some require addition of ethanol, and the carrier RNA must also be prepared. Perform these steps and label accordingly. This will be done once on opening a new kit of 250 extractions.
- Ensure samples and buffers are equilibrated to room temperature before proceeding with extractions
- You will need to set the pipette to the appropriate volume for each step. Carefully check this prior to beginning any step. You will be using the 1 mL pipette for most steps but will use the 100 uL pipette in Step 11.

Step 1: Add carrier RNA to Buffer AVL
Confirm that Buffer AVL has not precipitated. If it has, incubate at 80°C to dissolve. The amount to mix depends on how many samples you are extracting. Follow this table:

Number of Samples	Buffer AVL (mL)	Carrier RNA (in AVE) (uL)
1	0.56	5.6
2	1.12	11.2
3	1.68	16.8
4	2.24	22.4
5	2.80	28.0
6	3.36	33.6
7	3.92	39.2
8	4.48	44.8
9	5.04	50.4
10	5.60	56.0
11	6.16	61.6
12	6.72	67.2
13	7.28	72.8
14	7.84	78.4
15	8.4	84.0
16	8.96	89.6

Mix gently by inverting the tube 10 times. Do not vortex to avoid foaming.
This mixture should be prepared fresh each time, but it is stable at 2-8°C for up to 48h. Precipitate will form at this temperature, so it must be redissolved at 80°C. Do not warm this mixture more than 6 times, and do not incubate at 80°C for more than 5 min.

Step 2: Add 560 uL of Buffer AVL-Carrier RNA mixture into a new 1.5 mL tube. Repeat for the total number of samples being extracted. Change tips for each

Step 3: Add 140 uL of sample to the 1.5 mL tube. Change tips for each sample. Mix thoroughly by inverting/shaking or vortexing. Repeat for the total number of samples being extracted, adding 1 sample to each 1.5 mL tube.

Step 4: Incubate at room temperature for 10 minutes.

Step 5: Centrifuge tube(s) briefly.

Step 6: Pour some ethanol from the stock bottle to a small beaker for use in the following step. After completion, dispose of the ethanol and do not return this to the bottle. Add 560 uL ethanol (96-100%), and mix by pulse vortexing 15s or inverting/shaking. Briefly centrifuge.

Step 7: Apply 630 uL of solution from previous step to the QIAamp mini column in 2mL collection tube. Centrifuge 6000 xg (8000 rpm) for 1 min. Place the column in a clean collection tube.

Step 8: Repeat step 7, applying the remaining sample to the column.

Step 9: Add 500 uL Buffer AW1 to the column. Centrifuge 6000 xg (8000 rpm) for 1 min. Place column in clean collection tube and discard the used collection tube.

Step 10: Add 500 uL Buffer AW2 to the column and centrifuge at full speed (20 000 xg or 14 000 rpm) for 3 min.

Step 11: Place column in a clean 1.5 mL tube and add 60 uL Buffer AVE. Discard the used collection tube. Label with the sample ID. Incubate at room temperature for 1 minute.

Step 12: Centrifuge at 6000 xg (8000 rpm) for 1 min. This step elutes your RNA. Keep the elution (column flow-through) in the 1.5 mL tube, and discard the column. This tube will contain the RNA needed to progress to the RT-qPCR reaction setup. Use this directly in the test strips as outlined below in RT-qPCR Setup for your test format.

RT-qPCR Test Setup

Franklin Three9 Thermocycler

Tests come with one bead containing all reagents in each PCR tube. Nine samples can be run together per run. It is recommended to run a multiple of 3 at a time, but this is not necessary. Label the well for any tubes without patient samples as EMPTY when entering sample data, and for tracking. Do not cut strips of tubes or caps.

Once samples are extracted for all samples to be tested, transfer RNA from collection tubes test strips. Place the number of tubes that will be prepared for a given run into a PCR tube rack. Carefully re-seal the foil package containing the remaining tubes/strips.
Remove strip covering before adding extracted RNA.

Using a fixed or variable volume pipette provided move 20 µL of your extraction RNA directly to one PCR well containing lyophilized test(s).

- To do this, set the pipette to 20 µL (not required for fixed volume pipettes). Attach a disposable tip to the end of the pipette by pressing down firmly onto the tip while it's still in the box. Remove the pipette and tip.
- To move liquid, depress the top of the pipette down to the first stop, place the end of the tip into the liquid, and slowly ease up pressure on the top of the pipette until completely released. There should be no bubbles or gaps in the liquid at the end of the tip.
- To expel this liquid, open your PCR tube by peeling back the plastic covering, place the end of the pipette tip into the tube, close to the bottom but not touching the reagent beads, gently touch the pipette tip to the side of the tube, and depress the top of the pipette slowly and fully to the second stop. Pipette up and down 3-5 times to mix. No liquid should be left in the pipette tip and liquid should be in the bottom of the tube. Tap gently on a solid surface to bring liquid to the bottom of the tubes and to remove all bubbles. Once you have completed this for 3 samples, place the void filling cap onto the tubes. These will be loose, this is normal. Keep tubes upright. Repeat for additional samples to be tested in this run.
- Dispose of your pipette tip but not of the reusable fixed volume pipette.
- Do NOT label the tubes directly, as this may cause interference with the laser that reads fluorescence. Do carefully keep track of the samples and their orientation in your tray.

Once all samples are prepared, place them into your thermocycler. Use the step by step app to start a run with the “PBI SARS-CoV-2 Test Protocol”, in your instrument. The cycling conditions are:

55° C x 10 mins
95° C x 2 mins
45 cycles
95° C x 5 secs
60° C x 20 secs

Label all samples in the run, as well as tracking these in a second location. The app will prompt you to enter this data as you set up the run.

- Do not deviate from this protocol, edit or adjust it in any way
- The run time is approximately 1 hour
- Ensure that sample ID numbers entered correspond with recorded patient data, but do not reveal any personal identifiers in the sample name entered into the run

Maverick Thermocycler

Tests come with one bead containing all reagents in each PCR tube. Sixteen samples can be run together per run.

Once samples are extracted for all samples to be tested, transfer RNA to test tubes. Place the number of tubes that will be prepared for a given run into a PCR tube tray. Carefully re-seal the foil package containing the remaining tubes/strips. Remove tube covering before adding extracted RNA.

Using a fixed or variable pipette provided move 20 µL of your extraction RNA directly to one PCR well containing lyophilized test(s).

- To do this, set the pipette to 20 µL (not required for fixed volume pipettes). Then attach a disposable tip to the end of the pipette by pressing down firmly onto the tip while it's still in the box. Remove the pipette and tip.
- To move liquid, depress the top of the pipette down to the first stop, place the end of the tip into the liquid, and slowly ease up pressure on the top of the pipette until completely released. There should be no bubbles or gaps in the liquid at the end of the tip.
- To expel this liquid, open your PCR tube by peeling back the plastic covering, place the end of the pipette tip into the tube, close to the bottom but not touching the reagent beads, gently touch the pipette tip to the side of the tube, and depress the top of the pipette slowly and fully to the second stop. Pipette up and down 3-5 times to mix. No liquid should be left in the pipette tip and liquid should be in the bottom of the tube. Tap gently on a solid surface to bring liquid to the bottom of the tubes and to remove all bubbles. Once you have completed this for 3 samples, place the void filling cap onto the tubes. These will be loose, this is normal. Keep tubes upright. Repeat for additional samples to be tested in this run.
- Dispose of your pipette tip but not of the reusable fixed volume pipette.
- Do NOT label the tubes directly, as this may cause interference with the laser that reads fluorescence. Do carefully keep track of the samples and their orientation in your tray.

1. Once all samples are prepared, place them into your Maverick thermocycler. Run with the following cycling conditions:

   55° C x 10 mins
   95° C x 2 mins
   45 cycles
   95° C x 5 secs
   60° C x 20 secs

Choose the following fluorophore channels for each target:
UTR – FAM
E Gene – Texas Red
RNaseP – Cy5

Label all samples in the run, as well as tracking these in a second location.

- Do not deviate from this protocol, edit or adjust it in any way
- The run time is approximately 1 hour
- Ensure that sample ID numbers entered correspond with recorded patient data, but do not reveal any personal identifiers in the sample name entered into the run

CFX96 Thermocycler

Tests come with one bead containing all reagents in each PCR well of a 96-well plate.

To reconstitute the Precision Biomonitoring TripleLock SARS-CoV-2 Test for use, add 20 µL of purified RNA extract to a test well containing a reagent bead, and pipette up and down 3-5 times to mix fully. Change tips and repeat for each sample being tested. Ensure there are no bubbles. A plate spinner (not provided) can be used if desired to remove bubbles (seal the plate first). Once samples are each prepared, apply an optical adhesive seal to the plate and load into the thermocycler.

Enter cycling conditions as below:

55° C x 10 mins
95° C x 2 mins
45 cycles
95° C x 5 secs
60° C x 20 secs

Choose the following fluorophore channels for each target:
UTR – FAM
E Gene – Texas Red
RNaseP – Quasar 670

Label all samples in the run, as well as tracking these in a second location.

- Do not deviate from this protocol, edit or adjust it in any way
- The run time is approximately 1 hour
- Ensure that sample ID numbers entered correspond with recorded patient data, but do not reveal any personal identifiers in the sample name entered into the run

Precision Biomonitoring TripleLock SARS-CoV-2 Test Data Interpretation

After run completion, data should be interpreted using the table below.

E Gene	UTR	RNase P	Result
+	+	±	Positive
If only one target is positive		+	Inconclusive, repeat testing to confirm positive
If only one target is positive		-	Inconclusive, repeat testing to confirm positive
-	-	-	Invalid*
-	-	+	Negative

*If the RNaseP target is negative and the other targets are also negative, this means that there was an error in either extraction or cycling. If all the samples in the run have this result, it is likely a cycling error. If it is individual samples only, this indicates either a failed RNA extraction, or an insufficient amount of starting sample. Repeat your test.

Test results are considered negative for a target if the Cq value is above 38 cycles. For the Franklin thermocycler, results should be considered negative if the Cq value is above 38, or if the final maximum RFU of the curve is under 500.

If the PCR result is inconclusive for either of the viral targets in a given sample, a re-test of the patient or sample is suggested to determine if the patient is positive or negative.

A combination of Cq values and visual inspection of curves should be used to confirm sample positivity.

Controls

Positive and negative external controls are not provided with the device. Controls should be run according to any applicable regulations and the usual quality control procedures in your lab. Positive controls can be run as needed to assess ongoing performance, however the RNaseP target provides an internal control in each well for each test that confirms that the RNA extraction was successful, and that sufficient material was present for the test results to be valid. Microbix RED-S19-01 swab controls have been validated for use with this kit as a positive control and can be used per the manufacturer instructions. Nuclease-free water (e.g. IDT: 11-04-02-01) can be used as a negative control.

Warnings and Limitations

- Always use appropriate safety equipment and PPE. Consult SDS sheets for additional information.
- Dispose of any potentially biohazardous or otherwise harmful materials according the appropriate provincial and federal regulations in your region.
- Never add bleach directly to the M1 prep cartridge as this can create a dangerous compound.
- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- Laboratories are required to report all positive results to the appropriate public health authorities
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- False-negative results may arise from:
    - Improper sample collection or storage
    - Specimen collection after nucleic acid can no longer be found in the specimen matrix
    - Using unauthorized assay reagents
    - The presence of RT-PCR inhibitors
    - Mutation in the SARS-CoV-2 virus
    - Failure to follow instructions for use
- False-positive results may arise from:
    - Cross contamination during specimen handling or preparation
    - Cross contamination between patient samples
    - Specimen mix-up
    - RNA contamination during product handling
- This assay does not differentiate between SARS-CoV-2 and SARS-CoV-1
- The performance of this device has not been assessed in a population vaccinated against COVID-19

Technical Support

Email: support@precisionbiomonitoring.com
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