Test Kit Instructions: Wastewater Testing Using GeneCount™ SARS-CoV-2 RT- qPCR Detection Workflow

SECTION 1: RNA Concentration & Extraction

PROVIDED

- 15 mL Sterile Conical Tubes
- Lysis Buffer Concentrate
- Lysis Supplement 1A
- Wash Solution 1 Concentrate (Store at 25°C)
- Wash Solution 2 Concentrate (Store at 25°C)
- Elution Buffer NA (Store at 25°C)
- Nuclease Free Water
- Magnetic Beads (Store at 4°C)
- 1000 µL and 200 µL Filtered pipette Tips
- Wide mouth 1mL pipette tips (only for sample addition)
- 1.5 mL sterile disposable transfer pipettes

REQUIRED BUT NOT PROVIDED

- Tube Rack for 2 mL and 15 mL Tubes
- Permanent Marker
- Isopropanol* (95-100%)
- Ethanol (95-100%)
- Adjustable Volume 1000 µL and 200 µL Pipets

***Ethanol may be used as a replacement for isopropanol if it is not available.**

GETTING STARTED

- Wear safety glasses and disposable exam gloves.
- **Please read all reagent SDSs for instructions, hazards, and material safety.**
- Clean and set up a work area to process samples.
- Create "Wash Buffer 1" by adding **60 mL** of **Isopropanol** to **120 mL** of **Wash Solution 1 Concentrate**, per label instructions.
- Create "Wash Buffer 2" by adding **160 mL** of **Ethanol** to **40 mL** of **Wash Solution 2 Concentrate**, per label instructions.
- Rehydrate vial of **Lysis Supplement 1A** with **6.6 mL of Nuclease-Free Water**. Mix intermittently for 1 minute by swirling. Do not invert bottle.

Note: Solution may not fully dissolve.

RNA CONCENTRATION & EXTRACTION

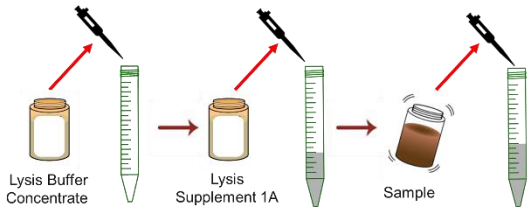
- Label a sterile 15 mL conical tube with identifying information for each sample.
- Add **6 mL** of Lysis Buffer Concentrate, **250 µL** of rehydrated **Lysis Supplement 1A**, and **1 mL** of **wastewater sample** using wide mouth 1mL pipette tips to each 15 mL sterile conical tube.

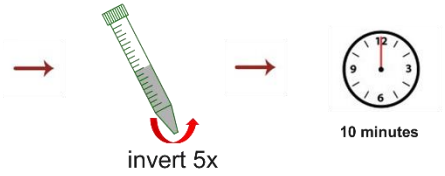
Note: Use wide mouth 1mL pipette tips only for addition of sample.

Note: Store unused Lysis Supplement 1A frozen at -20°C for up to 1 month in single-use aliquots to minimize freeze-thaws. Thaw completely before use.

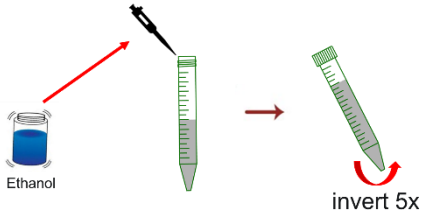
- Close cap and invert **5 times** to mix the sample solution**,** then incubate for **10 minutes**.

Note: If an incubator is not available room temperature can be used.



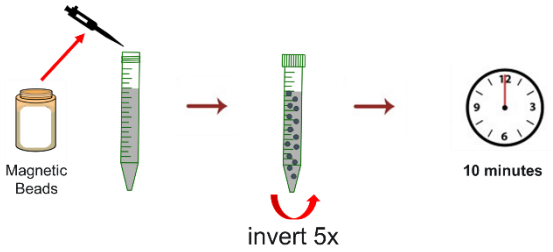


- After incubation, add **3.5 mL** of **Ethanol** and mix thoroughly by gently inverting the tube **5 times**.

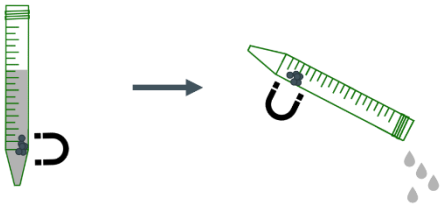


- Add **40 µL** of **Magnetic Beads** to the sample mixture. Invert the sample mixture 5 times to mix, then incubate again for **10 minutes**.

Note: Ensure that Magnetic Beads are fully resuspended by inverting 5 times before adding to the sample mixture. If an incubator is not available, room temperature can be used.



- After incubation, place in the magnetic rack to precipitate the Magnetic Beads on the side of the tube. Discard the supernatant.



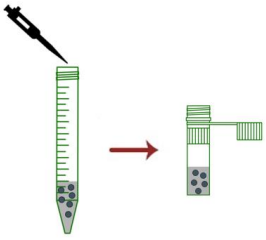
- Add **1 mL** of **Wash Buffer 1**. Cap and swirl the tube 10 times to mix.
- Place the tube in the magnetic rack to precipitate the Magnetic Beads. Discard the supernatant.
- Repeat the previous two steps with **Wash Buffer 1** twice more.

Note: Wash Buffer 1 is added and discarded a total of three times.

- Add **1 mL** of **Wash Buffer 2** to the Magnetic Beads. Cap and swirl the tube 10 times to mix.
- Place the tube in the magnetic rack to precipitate the beads and discard the supernatant.
- Repeat the above two steps with **Wash Buffer 2**.

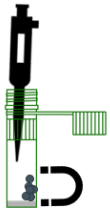
Note: Wash Buffer 2 is added and discarded a total of two times.

- Add **1 mL** of **Ethanol** to the Magnetic Beads. Cap and swirl the tube 10 times to mix then transfer the mixture to a new sterile 2 mL tube using a sterile disposable transfer pipette.



- Place the tube in the magnetic rack to precipitate the beads and discard the supernatant.

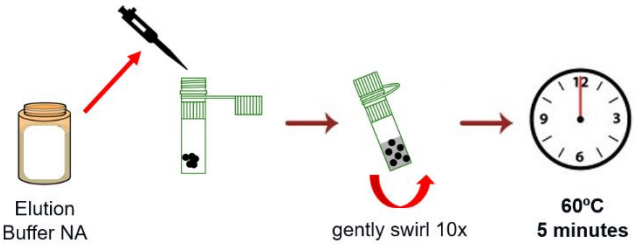
- Remove the tube from the magnet rack and allow the remaining pellet to pool in the bottom of the tube.
- Place the tube in magnet rack and allow the Magnetic Beads to accumulate to the side of the tube for 2 minutes. Remove the remaining pooled liquid at the bottom of the tube with a sterile 200 uL pipette without agitating the Magnetic Beads.



- Add **50 µL** of **Elution Buffer NA** to the **2 mL tube**. Cap and **gently** swirl the tube 10 times to mix.

Note: Ensure that the beads are resuspended in the elution buffer.

- Incubate the **Magnetic Beads** at **60°C** for 5



minutes.

- After incubation, apply the magnet to separate the **Magnetic Beads** and use a pipette to collect eluted RNA for analysis.

- Eluted RNA can be stored at -20°C

Note: If biobanking samples for longer than 2 weeks, store the eluted RNA at -80°C.

Note: Please read all reagent SDSs for instructions, hazards, and material safety

SECTION 2: qPCR Preparation

PROVIDED

- SARS-CoV-2 Advanced RT-qPCR Master Mix (Store at 25°C)
- PCR Strip Tubes
- Nuclease-Free Water (Store at 25°C)
- 20 µL Filtered Pipet Tips

REQUIRED BUT NOT PROVIDED

- Tube Racks for 1.5 / 2.0 mL and PCR Strip Tubes
- Adjustable Volume 20 µL Pipet

OPTIONAL BUT NOT PROVIDED

- Positive Control DNA (Lyophilized: Store at Ambient)

GETTING STARTED

- Wear safety glasses and disposable exam gloves.
- Clean and set up a work area to process samples.

INITIAL SETUP

- Gently tap **RT-qPCR Master Mix** bottle on a hard surface to collect contents at the bottom of the bottle.

  Note: Each bottle of RT-qPCR Master Mix contains enough for 48 samples.

- Remove and discard rubber stopper, and then transfer 825 µL of **Nuclease-Free Water** into the **RT-qPCR Master Mix** bottle.
- Recap and let **RT-qPCR Master Mix** rehydrate for 3 minutes. Mix occasionally by swirling. Do not invert bottle.

  Note: Rehydrated RT-qPCR Master Mix should be used immediately or frozen at – 20°C for up to 12 months. Avoid freeze-thawing the mix more than 3 times for best results.

- **Optional**: If using a positive control, transfer 50 µL of Nuclease Free Water into the Positive Control DNA tube. Recap tube tightly. Allow Positive Control to rehydrate for 5 minutes. Mix tube occasionally by inverting. Centrifuge for 5-10 seconds before use.

  Note: Rehydrated Positive Control should be used immediately or frozen at – 20°C for up to 12 months in single use aliquots to minimize freeze-thaws. Thaw completely before use.

ASSAY SETUP

- Thaw rehydrated **RT-qPCR Master Mix** if necessary.

  Note: Thaw on ice or on the benchtop. Avoid trying to speed up thawing by warming bottle in your hands.

- Dispense 15 µL of the Master Mix into each PCR Strip Tube.

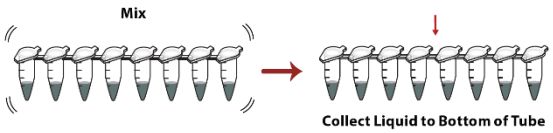
- Transfer 5 µL *of Nuclease-Free Water into the first PCR tube.* This is the **Negative Control**. Close tube.

  Note: Only one set of controls is needed per instrument run not per strip tube (if more than one is being used)

- Transfer 5 µL of each sample RNA into individual PCR tubes. Close each tube as sample is added. Leave the last PCR tube with no RNA if adding an optional positive control.
- **Optional**: Transfer 5 µL of the Positive Control DNA into the **last** PCR tube. This is the **Positive Control**. Close tube.

  Note: The Positive Control DNA is highly concentrated so care should be taken to not contaminate other samples or your work area.

- Gently mix each qPCR reagent tube.
- Using a robust downward motion, shake the contents of qPCR tubes to the bottom of tube.



Note: Be careful to note the correct orientation of the tubes to prevent accidentally reversing the tubes when inserting into the qPCR device. A small mark with a permanent marker on the side of the first tube can help prevent mis-orienting.

- Samples are now ready for analysis in a GeneCount™ Q-8, Q-16, or Q-96 device.

SECTION 3: Analysis on Q-Series Device

REQUIRED

- Q-8 or Q-16 qPCR Device
- GeneCount™ Software

OVERVIEW

The below procedure is based on using a GeneCount Q-8 device to run up to 7 samples plus a negative control or a GeneCount Q-16 to run up to 15 samples plus a negative control.

If using a GeneCount Q-96, please contact LuminUltra for alternative instructions.

GETTING STARTED

- Please visit https://www.luminultra.com/genecount/software to download the latest GeneCount™ instrument software
- Plug in qPCR Device to power outlet.
- Connect qPCR Device to computer via USB cable.
- Power on qPCR Device
- Open GeneCount™ software

Warning ⚠

When operating or performing work on the device, all relevant PPE guidelines should be followed taking special care to protect oneself and others from potentially contagious material.

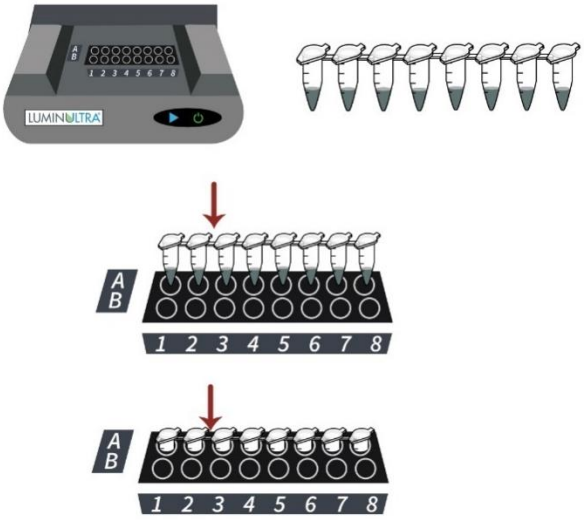
Note: Please read the GeneCount Q8 or Q-18 equipment manual for more instructions on safety and how to operate the qPCR Device.

qPCR Device precautionary Labels ⚠

	**This symbol indicates a safety alert**. Obey all safety messages that follow this symbol to avoid potential injury. If on the instrument, refer to the instruction manual for operation or safety information.
	**This symbol indicates an aperture from which potentially harmful light is emitted.** Do not peer into the LED light aperture/s.
	**This symbol indicates a potentially hot surface.** Avoid touching the marked surface when putting samples in or when lid is open

INITIAL SETUP

- Open latch on the front of the device and lift up lid gently.
- Place PCR strip tubes inside device, noting the coordinates of each sample.



- Close lid firmly until the latch is engaged.

SOFTWARE SETUP

- Chose “New Experiment” to start a new experiment or “Choose Template” if a template file of the experiment is already saved.

  Note: Check the top of the screen to confirm that the qPCR Device is connected (indicator = green). If it is not, close software, reconnect the qPCR Device, and reopen software.

- Enter in experiment name and all sample data in the corresponding sections by double clicking the appropriate box.

Field	Description
Name	Identity of the sample you are testing
Type	**Unknown:** The environmental sample you are testing **Negative control:** The assay mixed with **Nuclease-Free Water** **Positive control:** The assay mixed with the optional **Positive Control**
Quantity	Approximate volume of sample processed – for this method 1 mL. For the positive and negative control enter “20 µL”
Units	Use mL for liquid samples.
Extraction Method	This method is classified as a Lab Extraction.
Assay	Choose ‘SARS-CoV-2 Advanced’

- Click "Continue setup…" to view the program parameters.

  Note: These parameters have been pre-calibrated to suit the qPCR assay being run and do not need to be adjusted.

- Click "Start" and the qPCR program will begin.

- Upon completion of qPCR run, analyze data according to manufacturer's instructions to determine Ct values. Interpret data as outlined in below two tables.

SECTION 4: Result Interpretation

- Upon completion of qPCR run, analyze data to determine Ct values. Interpret data as outlined in below two tables.

Expected Performance of Controls		
Control Type	**SARS-CoV-2**	**Internal Control**
Positive (Optional)	PASS	PASS or FAIL*
Negative	PASS	PASS

*Positive controls or positive samples may result in the internal control not amplifying properly. This is normal. The internal control is only important for negative controls and negative samples.

Interpreting Sample Results		
SARS-CoV-2	**Internal Control**	**Result**
DETECTED	PASS or FAIL*	SARS-CoV-2 Detected
BELOW DETECTION LIMIT	PASS	SARS-CoV-2 Not Detected
BELOW DETECTION LIMIT	FAIL	Invalid

Quantitative results will be provided in test results and expressed as units of copies/mL.

TROUBLESHOOTING

Issue	Recommendation
I would like to process a different sample type than that recommended for this test kit.	Please contact LuminUltra to discuss your sample type. Additional procedures and test kits are available.
The negative control came up positive for SARS-CoV-2.	Try re-running the assay in a cleaner location and keep all qPCR reagents separate from the extraction process.
The positive control was not detected.	1. Check the assay file to see that the correct microbe was chosen from the "Assay" drop down menu. 2. Ensure that the positive control is being stored properly 3. Check to see if the positive control has expired.
A sample that came up negative for SARS-CoV-2 also showed a failed internal control.	The sample may contain residual inhibitors: 1. Verify that the RNA extraction procedure was followed correctly 2. Dilute extracted RNA with water 1:5 or 1:10 and rerun the qPCR..

Note: All reagents used in this test have a 12-month shelf life.

Note: For Research Use Only. Not for use in human or veterinary diagnostic procedures.

ORDERING INFORMATION

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