Field Test Kit Instructions - GeneCount™ LT qPCR Assay Kit for Legionella species

DNA EXTRACTION

PROVIDED

- Sterile 50mL (or 60mL) Syringe (24)
- Sterile 3 mL Syringe (24)
- 5 mL Tubes w/LT Lysis Buffer A (24)
- LT Wash Buffer A (500 mL Bottle)
- DNA Clean-Up Column 1 w/Collection Tube (24)
- DNA Elution Tube (24)
- Syringe Filter (24)
- Extension Tip (24)

REQUIRED BUT NOT PROVIDED

- Tube Rack for 1.5/2.0 mL and 5 mL Tubes
- CAPPRondo CR-68X Microcentrifuge
- Fixed Volume 100µL Pipet
- Safety Glasses
- Disposable Gloves
- Disposable Anti-Bacterial Face Mask

COMPATIBILITY

This procedure is designed for low solid fluid samples.

GETTING STARTED

- Wear safety glasses and face mask
- Put on gloves and set up work area.

1. PROCESS SAMPLE

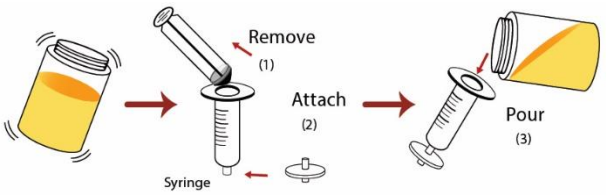
- Refer to the Kit Volume Recommendations chart for the recommendations for your sample type.

Process or Surface Water Volume

Recommendations

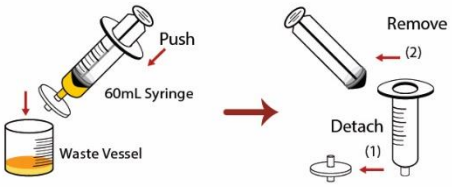
Lowest Detectable Concentration (GU/ml)	Recommended Volume (mL)
100	50
10	250
2.5	1000
1	2500

- Mix the sample well.
- Remove the plunger from a new 50mL (or 60mL) syringe and attach a new syringe filter.
- Pour 50 mL of the sample into the syringe.



- Re-insert the plunger and slowly push the sample through the filter into a waste receptacle.
- Detach the filter and gently remove the plunger from the syringe with a twisting motion.

Note: Species of Legionella can be dangerous when aerosolized, use caution when removing the plunger.



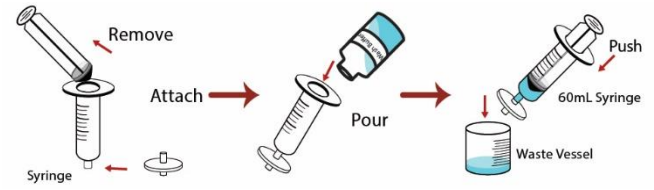
- Repeat previous steps until the recommended sample volume has been pushed through the filter.

Note: Record the actual volume of sample processed.

2. FILTER WASHING & DRYING

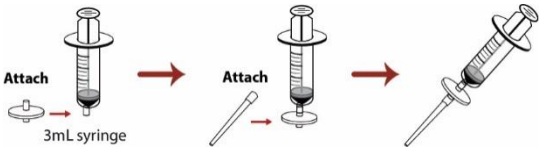
- Re-attach the filter to the 50mL (or 60mL) syringe barrel and pour 20 mL of **LT Wash Buffer A** into the syringe barrel. Re-insert plunger and pass the Wash Buffer slowly through the filter and collect into waste receptacle. Push remaining air in syringe barrel through the filter to dry.

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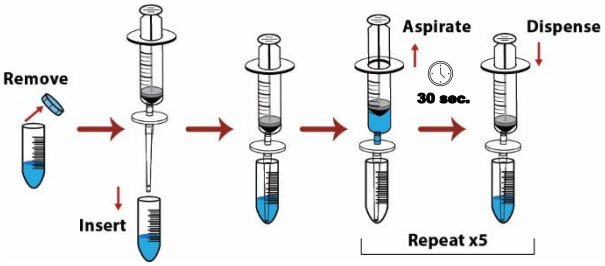
3. EXTRACTION

- Transfer the syringe filter to a new 3 mL syringe. Attach provided extension tip to the outlet end of the syringe filter.



- Lower the extension tip connected to the syringe filter into a tube of **LT Lysis Buffer A**.
- Slowly pull as much Lysis Buffer as possible through the filter by gently pulling up on the syringe plunger. Wait 30 seconds.

  Note: If Lysis Buffer shows evidence of precipitation before use, warm tube in hand until components are re-dissolved before using.
- Depress the plunger slowly to dispense the Lysis Buffer back into the same Lysis Buffer tube.
- Repeat the prior 2 steps 4 additional times. *Be careful to pull and depress the plunger slowly to avoid creating excessive foam in the tube or syringe.*



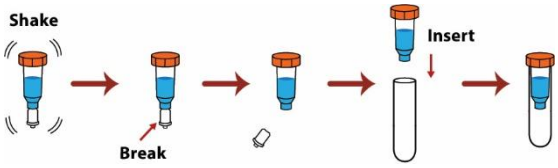
- Slowly dispense all Lysis buffer back into the same Lysis Buffer tube.

4. DNA CLEANUP

- Resuspend the contents of a new **DNA Clean-Up Column 1** by shaking the tube vigorously for 5-10 seconds, then settle the tube contents by gently tapping the tube on the bench.

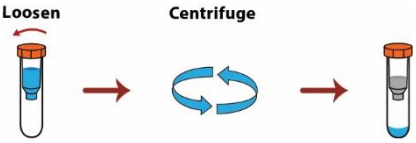
Note: Allow cold columns to warm up to room temperature before use.

- Bend the column tip to break it, then discard tip.
- Place the column into the provided 2 mL **Collection Tube**.

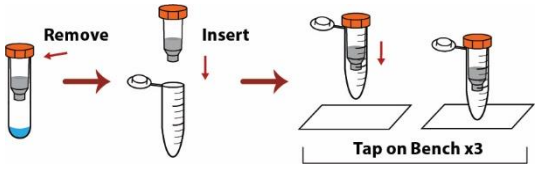


- Loosen the column cap slightly and then centrifuge the column with collection tube for **1 minute** at **3000 RPM** (~ 500x g) to remove storage buffer.

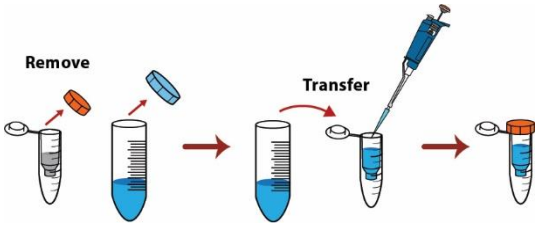
Note: Always be sure to balance centrifuge.



- Place the column in a new empty 1.5 mL **Elution Tube**.
- Tap column in elution tube firmly on the bench three times to ensure resin is compacted tightly.



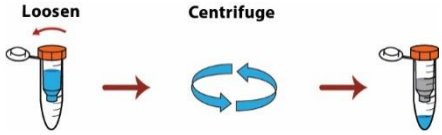
- Slowly apply 100uL of the lysed sample to the top of the compacted resin bed. Take care not to touch or disturb the resin.



- Loosen the column cap slightly and centrifuge the column with elution tube for **2 minutes** at **3000 RPM** (~ 500x g).

Note: Always be sure to balance centrifuge.

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- The purified DNA is in the Elution Tube and is ready for immediate qPCR analysis. Discard column after use.

qPCR ASSAY PREPERATION

PROVIDED

- Lyophilized qPCR Reagents (4-Well Strips) (12)
- Positive Control DNA Tube (12)
- Nuclease-Free Water Tube (12)
- 100 µL Filtered Pipet Tips (Box of 96)

REQUIRED BUT NOT PROVIDED

- Tube Racks for 1.5 / 2.0 mL and PCR Strip Tubes
- Fixed Volume 100 µL Pipet
- Fixed Volume 20 µL Pipet
- Safety Glasses
- Disposable Gloves

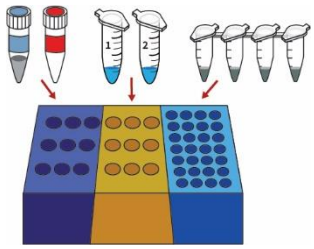
GETTING STARTED

- Wear safety glasses.
- Put on gloves and set up work area.

INITIAL SETUP

- Arrange the below reagents into appropriately sized sections of your tube rack(s):
  - Nuclease-Free Water
  - Positive Control DNA
  - DNA Samples purified using the GeneCount™ LT DNA Purification Kit
  - Lyophilized qPCR reagents

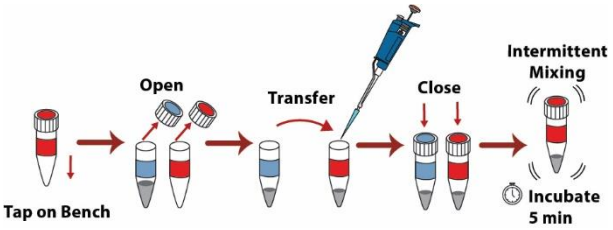
Example:



- Retrieve the Positive Control DNA tube and gently tap the bottom of the tube on the bench to collect the colored pellet to the bottom of the tube.

Note: The colored pellet is very small so it may not be easily visible by eye.

- Using the Fixed volume 100 µL Pipet, transfer 100 µL of Nuclease-Free Water to the Positive Control DNA tube. Recap the tube, then gently tap on the bench to collect the droplets, and then allow to rehydrate on the bench for 5 minutes, mixing occasionally by gently swirling the tube.

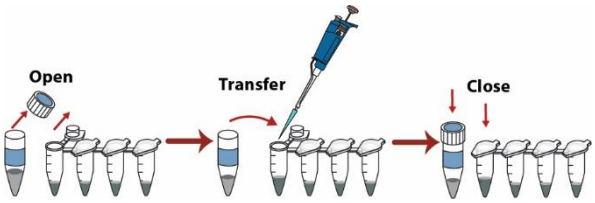


Note: While the rehydrated Positive Control DNA tube is incubating on the bench, you can use this time to begin setting up the assays as outlined in the next step.

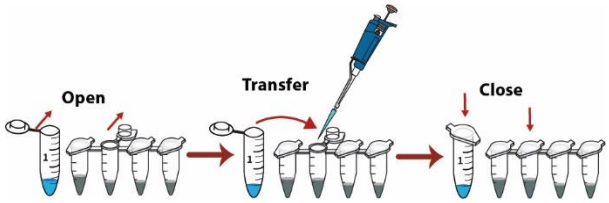
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ASSAY SETUP

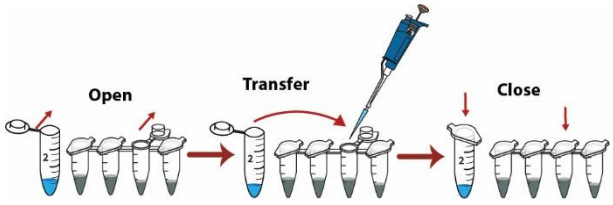
- Dispense the rehydrated reagents and samples into the appropriate assay tube, working from Left to Right.
- Using the Fixed volume 20 µL Pipet, transfer 20 µL of Nuclease-Free Water into the **first** qPCR reagent tube. This is the Negative Control. Recap both tubes.



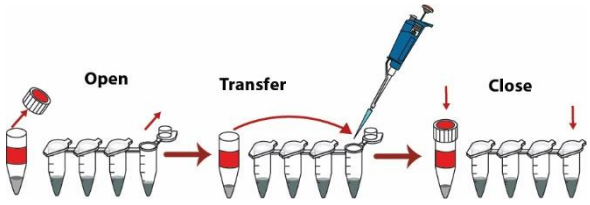
- Transfer 20 µL of the first DNA sample into the **second** qPCR reagent tube. Recap both tubes.



- Transfer 20 µL of the second DNA sample into the **third** qPCR reagent tube. Recap both tubes.



- Transfer 20 µL of the rehydrated Positive Control DNA into the **fourth** qPCR reagent tube. This is the Positive Control. Recap both tubes.

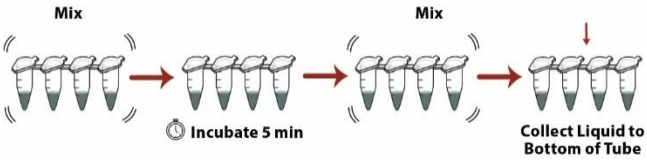


Note: The Positive Control DNA is highly concentrated so care must be taken to not contaminate other samples with the Positive Control DNA to prevent inaccurate results.

- Vigorously mix each qPCR reagent tube. Then let reagents sit on bench for 5 minutes. Vigorously mix each qPCR reagent tube again.

Note: While the qPCR reagent tubes are incubating on the bench, you can use this time to setup the GeneCount™ Q-8 or Q-16 software.

- Using a robust downward motion, shake rehydrated 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.

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

Note: Additional DNA samples can be added to additional lyophilized qPCR strip tubes and analyzed at the same time.

Q-8 or Q-16 Analysis

PROVIDED (purchased separately)

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

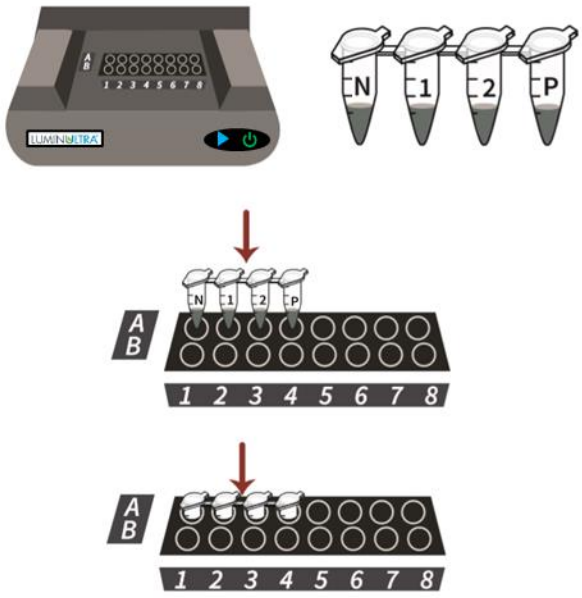
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GETTING STARTED

- Plug in qPCR Device to power outlet
- Connect qPCR Device to computer via USB cord (unless it is the touchscreen model)
- Power on qPCR Device, then open GeneCount™ Software

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 Assay” 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 and recognized. If it is not, close software, reconnect the qPCR Device, and reopen software.

- Enter in experiment name and all sample data in the corresponding coordinates by double clicking the appropriate box.
- Click “Continue setup…” to view the program parameters.

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

- Click “Start” and the qPCR program will start running automatically.

Results Analysis

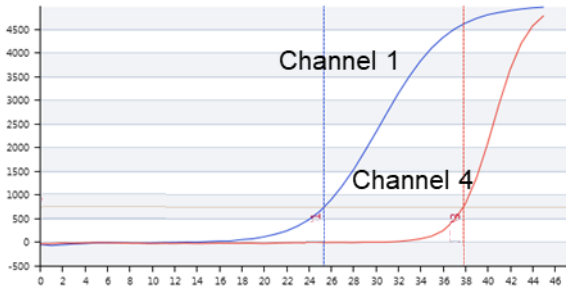
Graph

Field	Description
Name	The identity of the sample you are testing
Type	Unknown: The environmental sample you are testing Negative control: The assay resuspended with Nuclease-Free Water Positive control: The supplied positive control
Quantity	Amount of environment sample processed. For the positive and negative control enter “20 uL”
Units	Use mL if filtering a volume, grams for solid samples, or cm^2 for swabbed biofilms
Extraction method	Please refer to the protocol used when processing the sample to determine if it was a field extraction or a lab extraction
Assay	The microbe being screened for by the qPCR assay

- The assay monitors the concentration of Legionella by observing a signal that detects Legionella DNA. The more DNA that is present in the sample the earlier the signal will be detected. The point at which the signal is detected is called the Cycle Threshold (Ct)

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- For this assay, the qPCR device will monitor two different signal channels. If you are using the Q-8 or the Q-16 these are Channel 1 and Channel 4.
- Channel 1 detects Legionella DNA and is represented by a blue line.
- Channel 4 detects an internal positive control which can be used to troubleshoot possible issues in the assay and is represented by a red line.



Concentration

- After the qPCR run has finished the concentration of the sample will display under the “Analysis” tab. This result takes into account the amount of sample processed and how the sample was extracted.
- The results are given as Genomic Units (GU), which in this qPCR assay are based on each Legionella bacteria having one genomic unit. This value is similar to Colony Forming Units (CFU), but it is NOT EQUVILENT.

Note: Most governmental and regulatory bodies detect concentrations of Legionella in CFU.

Interpretation Matrix

	Internal Control	
Legionella sp.	Pass	Fail
Detected	The assay has worked as intended and there is a detectable concentration of Legionella DNA in the sample.	Legionella detection can sometimes cause the internal control to fail, but the detected concentration is still valid.
Below Limit of Detection	The assay has worked as intended and there is not a detectable concentration of Legionella DNA in the sample.	This assay may have been inhibited. Purify another 100 ul from the sample lysate and re-run. If the problem persists, dilute the sample 1:10 with clean water before extracting.

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5. TROUBLESHOOTING GUIDE

Issue	Recommendation
Difficult or impossible to push recommended volume of fluid sample through syringe filter.	1. Some samples have a high concentration of solids and the recommended volume cannot be filtered efficiently. In this case, record the actual volume that was filtered and continue with the protocol. Note: This may reduce the sensitivity of the procedure. 2. Contact LuminUltra to determine if another protocol or filtration method may be better suited for your testing in the future. 3. If a large volume of sample will be routinely filtered (e.g. for drinking water) then using a vacuum pump and manifold could be a more efficient alternative to the syringe.
My fluid sample has some oil in it and an emulsion forms during the lysis step. What should I do?	An alternative wash buffer may be required for this type of sample. Please contact LuminUltra to discuss options.
The final elution volume of purified DNA from column is lower than normal.	1. Make sure cap is slightly loose on column before centrifuging. 2. Centrifuge column in elution tube for an additional 1 minute.
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.
How much purified DNA will I have at the end of this procedure?	You will typically collect ~ 100 µL of purified DNA. This is enough to run 4-5 qPCR assays based on the standard volume used per assay (20 µL).
I need additional purified DNA to run many qPCR assays. Can I load more lysate onto the DNA Clean-Up Column 1?	Do not load more than the recommended volume of lysate onto the column. However, additional columns may be used to process more of the lysate.
I cannot fit all of the columns I'm processing into the centrifuge at the same time.	Up to 4 columns will normally fit conveniently in the CAPPRondo CR-68X Microcentrifuge at one time. Reload the centrifuge to process additional samples.
The negative control was detected.	The LuminUltra Legionella species assay is designed to be very sensitive and cross-contamination of the extracted sample can cause small amounts of DNA to be present in the negative sample. 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.

ORDERING INFORMATION

- **LuminUltra Technologies Ltd.**
  520 King Street, Fredericton, NB,
  Canada, E3B 6G3
- **LuminUltra Technologies Inc**.
  1448 South Rolling Road, Suite 018,
  Baltimore, MD, USA, 21227
- **LuminUltra Technologies SAS**
  Paris Montparnasse Business Centre
  140 bis rue de Rennes,75006 Paris

  Tel: +1 (506) 459 8777

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