Instructions for Use

LifeDireX COVID-19 RT-qPCR Kit

PRODUCT NAME
LifeDireX COVID-19 RT-qPCR Detection Kit
Batch Number: LifeDireX - QP019-0100

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1. Intended Use

The LifeDireX COVID-19 RT-qPCR Detection Kit is a real-time RT-qPCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 for human respiratory tract specimens (such as nasal swabs, mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) from individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper and lower respiratory specimens 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 infective status. The agent detected may not be the definite cause of disease. Positive results do not rule out bacterial co-infection with other viruses. 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. The LifeDireX COVID-19 RT-qPCR Detection Kit is intended for use by qualified trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

2. Principle of Detection

This product is a fluorescent probe-based RT-qPCR assay system. Firstly, the RNA of SARS-CoV-2 will be reverse transcribed into cDNA by reverse transcriptase, and then PCR amplification will be performed with cDNA as template. During amplification of the template, the probe will be degraded due to the 5´-3´ polymerase activity and exonuclease activity of DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The N gene of SARS-CoV-2 will be detected qualitatively by FAM channel, the RdRp gene of SARS- CoV-2 will be detected qualitatively by ROX channel, and the internal control RP (human gene) will be detected by HEX channel. Internal control is used in the kit for quality control starting from sample collection.

Instruments: Bio-Rad CFX96™ Real-Time PCR Detection System.

3. Kit Contents

Table 1. LifeDireX COVID-19 RT-qPCR Kit Contents

Component	Description	Amount Supplied (per 100 rnxs)
2X qPCR MasterMix	Multiplex assay primers/probes for N, RdRp and RP (human) genes with Hot Start DNA polymerase, dNTPs & buffer	1000µL x 1
RScript Enzyme Mix	RScript reverse transcriptase with RNase inhibitor	40µL x 1
Positive Control	Pseudo-virus DNA containing N and RdRp genes	50µL x 1
Negative Extraction Control	Cancer cell line with RP gene	1mL x 1
Nuclease-Free Water	DEPC-treated water	1mL x 1

4. Storage and Handling Requirements

- LifeDireX COVID-19 RT-qPCR detection kit is shipped with dry ice and gel packs.
- All components of the kit arrive in solution.
- All components of the kit must be stored at -20°C upon arrival.
- Do not use kit components after expiration date printed on the box label.
- If there is damage to the packaging inside, outside or kit contents have been tempered with, or storage condition failed to meet above -20°C, do not use.
- Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.
- Repeated freezing and thawing may lead to inaccurate results.
- The kit is stable for up to 1 year from date of release.

Note: Inaccurate results may be obtained if the kit is not handled according to the instructions provided.

5. Product Description

2X qPCR Master Mix: It is a multiplex assay containing primers/probes for N, RdRp and RP (human) genes with Hot Start DNA polymerase, dNTPs & buffer.

RScript Enzyme Mix - Unique blend of RScript reverse transcriptase with RNase inhibitor.

Positive Control: Ensures the assay is performed according to its use by evaluation with Pseudo-virus DNA containing N and RdRp genes.

Negative Extraction Control: Refers to the cancer cell line with RP gene.

Nuclease-Free Water: DNase, RNase, and nuclease-free, as in reference to DEPC-treated water

6. Quality Control

In order to evaluate the quality control, the test includes Positive and Negative controls. They might also be used for laboratory verification.

Table 2. Positive and Negative Control

Products of quality control	Requirements of Quality Control		
	FAM Channel	HEX Channel	ROX Channel
Positive Control of SARS-COV-2	Ct<35	Undetected	Ct<35
Negative Extraction Control	Undetected	Ct<35	Undetected

7. Limitations

- This kit should be transported under 4°C. We are unable to demonstrate the kit quality if the temperature while transporting is upper 37°C.
- Improper sample collection, shipping and storage may cause false-positive.
- Detection may be affected by sample collection methods and the stage of infection.
- Always use new pipette tips with aerosol barriers.

8. Warning and Precautions

- The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.
- Quickly prepare the reaction mix on ice or in the cooling block.
- The decrease of detection effect even the false negative result may occur if there are any mistakes in the transportation, storage and operation of reagents. SARS-CoV-2 early infection or other respiratory virus infection cannot be excluded in patients with negative results.
- For in vitro diagnostic use.
- For prescription use only.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- Handle all specimens as if infectious and use safe laboratory procedures.
- Inappropriate sample collection, transfer, storage and operation may lead to inaccurate test results.
- RNA extraction shall be carried out as soon as possible after sample collection to avoid degradation.
- The disposal of this kit will not cause any special risk or harm.

9. Protocol

1. **Prepare the PCR Reaction:** Thaw and assemble the following components in a 0.2 ml PCR tube on ice just prior to use: 2X qPCR MasterMix and RScript Enzyme Mix.
   Caution: Do not add more than one RNA sample into a single qPCR tube. Mix gently. If necessary, centrifuge briefly.

Table 3: PCR reaction preparation components

Component	Sample(s)	Positive Control	No Template Control	Negative Extraction Control
RNA Sample[1]	5 µl	-	-	-
2X qPCR MasterMix	10 µl	10 µl	10 µl	10 µl
RScript Enzyme Mix	0.4 µl	0.4 µl	0.4 µl	0.4 µl
Positive Control Template	-	5 µl	-	-
Negative Extraction Control	-	-	-	5 µl
Nuclease-free H_2O	4.6 µl	4.6 µl	9.6 µl	4.6 µl
Total Volume	20 µl			

2. Prepare the Controls: Use the Nuclease-free H2O for the No Template Control while using Positive Control for the Positive Control setup. Cap tubes tightly and place them in the thermal cycler.

3. Run Method: Set the thermal cycler for 45 cycles as follows:

Table 4. Steps and cycles

Steps	Temperature	Time	Cycle(s)
cDNA Synthesis	42°C	15 minutes	1
Pre-Denaturation	95°C	5 minutes	1
Denaturation	95°C	10 seconds	45
Annealing	60°C	60 seconds	

Note: Optimal conditions for amplification will vary depending on the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

4. Detection: As three channels (FAM, ROX, HEX) are used in this one tube qPCR assay, we recommend to perform the channel calibration as requested by its manufacturer. Please refer to the instrument's user manual to perform this calibration. Choose the FAM, ROX, and HEX channels for each sample to be tested with the LifeDireX COVID-19 RT-qPCR Detection Kit. Select "None" for ROX passive reference on any qPCR machine requiring ROX as the reference dye.

Table 5. Expected Performance of Controls

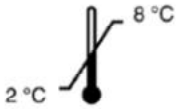
Control Type	Used to Monitor	Expected Results and Ct Values		
		N (FAM)	RdRP (ROX)	RP (HEX)
No Template Control	Assay or extraction reagent contamination	Negative Ct ND	Negative Ct ND	Negative Ct ND
Positive Control	Improper assay setup and reagent failure, including primer and probe degradation	Positive Ct < 35	Positive Ct < 35	Negative Ct ND
Negative Extraction Control	Cross-contamination during extraction	Negative Ct ND	Negative Ct ND	Positive Ct < 35

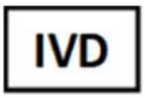
Functionality of the reaction mix

Table 6. Interpretation of Results

SARS-CoV-2			Interpretation	Action
N	RdRP	RP		
+	+	+/-	Positive	Report result to health authority.
If only one of the two targets are positive		+/-	Inconclusive Result	Repeat RT-qPCR of samples or repeat from extraction step. If result is still inconclusive, recommend collection of new specimen(s) from the patient.
-	-	+	Negative	SARS-CoV-2 not detected. Report result to health authority
-	-	-	Invalid Result	Repeat from extraction step. If the repeated result remains invalid, recommend collection of a new specimen(s) from the patient.

10. Symbols used in Packaging

Symbol	Used for	Example of Usage
	Temperature limit	
	Use-by date	
	Batch code	
	Catalog number	
	Manufacturer	
	Date of Manufacture	

Symbol	Used for
	Caution
	Consult instructions for use
	Research use only
	CE mark
	*In vitro* diagnostic medical device
	Authorized representative in the European Community
	Positive control
	Keep away from sunlight
	Prescription Use Only

11. Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantifying nucleic acid targets with the kit.

Table 7. Solutions for troubleshooting

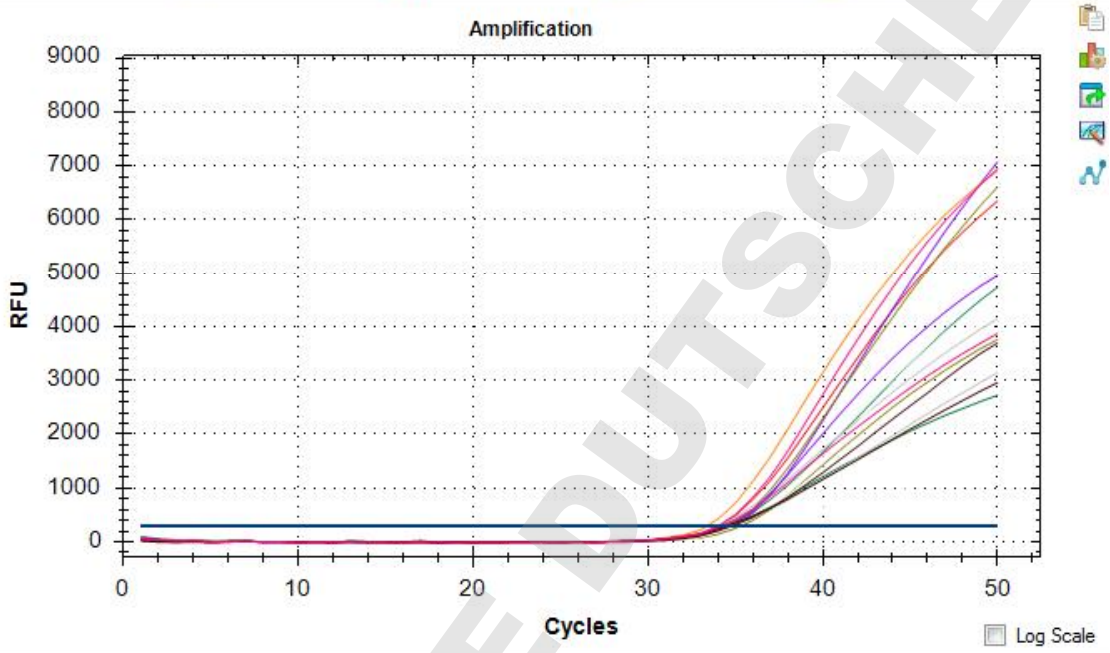
Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 15% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 15% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
Low or High Reaction Efficiency	Insufficient Optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions.

12. Performance Characteristics

Analytical Sensitivity and Limit of Detection (LOD)

A study was performed to assess the performance of LifeDireX COVID-19 RT-qPCR Kit. A testing of 20 replicates at the tentative limit of detection (LoD) concentration was carried out and it was further confirmed by testing 20 additional replicates of samples at 10 copies/reaction n1 gene by spiking in.

Table 8. Limit of Detection LifeDireX COVID-19 RT-qPCR Kit.



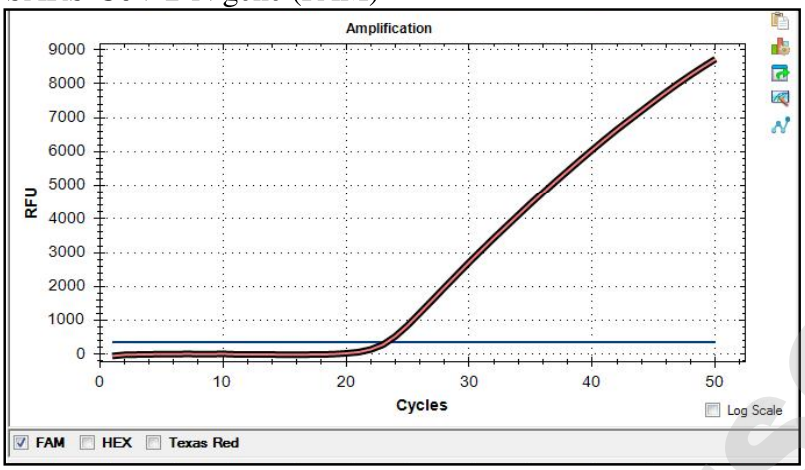
Target	Concentration	Detection Rate	Mean Ct
SARS-CoV-2-N gene	10 copies	100 % (20/20)	34.40

Typical S-Shape Amplification Curve

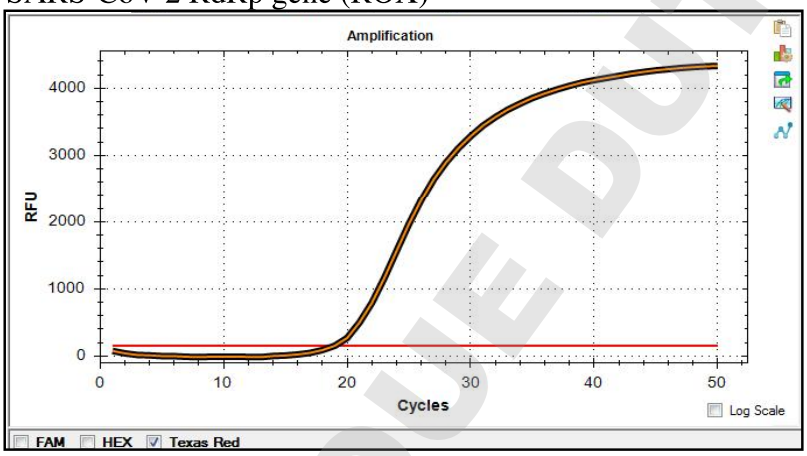
Considering the performance difference in real-time PCR instruments, thresholds for three fluorescence signals (FAM, ROX, and HEX) are set manually by the operator.

Table 9. The Typical S-shape Amplification Curve Example

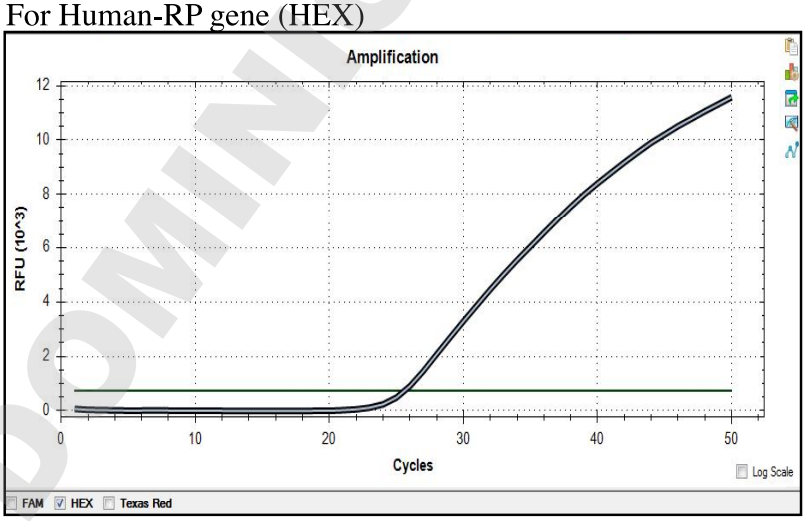
SARS-CoV-2-N gene (FAM)



SARS-CoV-2 RdRp gene (ROX)



For Human-RP gene (HEX)



13. Method of Sterilization

Sample Collection, Storage and Transport

- Flocked swabs are preferred. Sterile dacron or rayon swabs with plastic or flexible metal handles may also be used. Do NOT use cotton or calcium alginate swabs or swabs with wooden sticks as they may contain substances that inactivate viruses and inhibit PCR.
- Always use sterile pipette tips with filters.
- Use 15% bleach to sterilize the environment.