KIT HANDBOOK

MAY 2020

DÉTECTION EXPERT 1S
SARS COV-2

ONE STEP REAL TIME REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION KIT FOR QUALITATIVE DETECTION OF SARS COV-2
[5 Channel Format]

V2.2.

CE IVD

QUALITATIVE IN-VITRO DIAGNOSTIC

Σ 100
[Cat. No. GS.D1F.31519.2.100]

Σ 1000
[Cat. No. GS.D1F.31519.2.1000]

GeneStore France SAS
800 Avenue Du Chateau Du Jouques,
Gemenos, 13420, France

REF GS.D1F.31519.100
GS.D1F.31519.1000

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MAT GS.D1F.31519.2.HB.EN.V.2.2.

GENESTORE
FRANCE

COMMITTED TO HIGH QUALITY IN NUCLEIC ACID TECHNOLOGIES

GeneStore is a genomics diagnostics focused company head-quartered in Provence, France.

GeneStore presently operates R&D and manufacturing facilities across Europe, Middle-east, South America, and South Asia.

We strongly believe in providing advanced, and high quality products across the globe to ensure a simplified and robust sample to result procedure.

For more information on GeneStore visit: www.genestore.org.

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INTENDED USE

The GeneStore Detection Expert 1S © SARS CoV-2 kit is a one step real time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of SARS CoV-2 viral nucleic acid in upper and lower respiratory specimens (such as nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL)) 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 nasopharyngeal swabs in transport media 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.

Testing with the Detection Expert 1S © SARS-CoV-2 RT-PCR test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

PRODUCT DESCRIPTION

The GeneStore Detection Expert 1S © SARS CoV-2 kit includes assays and controls for a RT-PCR test for the qualitative detection of RNA from SARS COV-2 in nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

GeneStore Detection Expert 1S © SARS CoV-2 includes the following components:

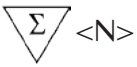
GeneStore Detection Expert 1S © SARS CoV-2 rRT-PCR Kit

A. SARS CoV-2 Real Time PCR multi-plex assay containing two primer and probe sets to detect two regions in the N gene of the SARS-CoV-2 genome, and

B. One primer probe set to detect the human RNase P (RP) in a clinical sample.

C. Positive controls containing targets specific to the SARS CoV 2 genomic regions covered by the assay.

Symbols



Contains reagents sufficient for <N> tests

Use by

IVD — In vitro diagnostic medical device

REF — Catalog number

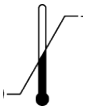
LOT — Lot number

MAT — Material number

COMP — Components

CONT — Contains

NUM — Number



Temperature limitation

Manufacturer

Consult instructions for use

Important note

Reagent Storage, Handling, and Stability

1. Store all dried primers and probes and the positive control, nCoVPC, at 2-8°C until re-hydrated for use. Store liquid materials between -25°C to -15°C.

2. Always check the expiration date prior to use. Do not use expired reagents.

3. Protect fluorogenic probes from light.

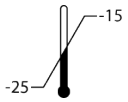
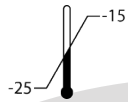
4. Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on a cold block at all times during preparation and use.

5. Do not refreeze probes.

6. Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.

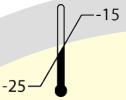
KIT CONTENTS

Detection Expert SARS CoV-2 © One Step rRT PCR Kit 100 Reactions
[Cat No. GS.D1F.31519.2.100]

Component	Description	Quantity	Storage Conditions
Detection Expert 1S © SARS CoV-2 One Step rRT PCR Kit 100 Reactions **CAT. NO.** GS.D1F.31519.2.100	SARS CoV 2 PROBE MIX • N1 [FAM] • N2 [HEX] • RNaseP [CY5]	463.50 µL	 -25°C to -15°C
	Probe Expert© One Step RT PCR ENZYME MIX	1236.00 µL	
Detection Expert © SARS CoV-2 Control Pack Cat. No. GS. REFMA. QUAL.31519.2.100	Positive Control SARS CoV 2 N1 + N2	20.00 µL	 -25°C to -15°C

KIT CONTENTS

Detection Expert SARS CoV-2 © One Step rRT PCR Kit 1000 Reactions
[Cat. No. GS.D1F.31519.2.1000]

Component	Description	Quantity	Storage Conditions
Detection Expert 1S © SARS CoV-2 One Step rRT PCR Kit 1000 Reactions Cat. No.: GS.D1F.31519 .2.1000	SARS CoV 2 PROBE MIX • N1 [FAM] • N2 [HEX] • RNaseP [CY5]	463.50 µL [10 Tubes]	 -25°C to -15°C
	Probe Expert© One Step RT PCR ENZYME MIX	1236.00 µL [10 Tubes]	
Detection Expert © SARS CoV-2 Control Pack Cat. No. GS.REFMA. QUAL. 31519.1000	Positive Control SARS CoV 2 N1 + N2	100.00 µL	 -25°C to -15°C

REQUIRED MATERIALS NOT SUPPLIED

1. Vortex mixer
2. Microcentrifuge
3. Micropipettes (2 or 10 µL, 200 µL and 1000 µL)
4. Multichannel micropipettes (5-50 µl)
5. Racks for 1.5 mL microcentrifuge tubes
6. 2 x 96-well -20µC cold blocks
7. Real-Time PCR Systems with analysis software
8. Nucleic Acid Extraction Systems
9. Molecular grade water, nuclease-free **(to be used as a negative control)**.
10. 10% bleach (1:10 dilution of commercial hypochlorite bleach)
11. DNAZapTM (Ambion, cat. #AM9890) or equivalent
12. RNAse AwayTM (Fisher Scientific; cat. #21-236-21) or equivalent
13. Disposable powder-free gloves and surgical gowns
14. Aerosol barrier pipette tips
15. 1.5 mL microcentrifuge tubes (DNase/RNase free)
16. 0.2 mL PCR reaction plates/strip tubes
17. Optical 8-cap Strips (if using strip tubes)

INSTRUMENT USED WITH THIS KIT

This kit has been validated for use with the following instruments:

- Mx 3005P™ QPCR System (Stratagene)
- ABI Prism® 7500 SDS (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Dx System (Bio-Rad)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- QuantStudio 5 Real-Time PCR (Applied Biosystems)

WARNINGS & PRECAUTIONS

1.For in-vitro diagnostic use (IVD).

2.Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.

3.Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.

4.Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.

5.Specimen processing should be performed in accordance with national biological safety regulations.

6.If infection with 2019-nCoV is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

7.Performance characteristics have been determined with human upper respiratory specimens and lower respiratory tract specimens from human patients with signs and symptoms of respiratory infection.

8. Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).

9. Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.

10.Amplification technologies such as PCR are sensitive to accidental introduction of PCR products from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.

Continued on next page.

WARNINGS & PRECAUTIONS

10.1.Maintain separate areas for assay setup and handling of nucleic acids.

10.2.Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.

10.3. Change aerosol barrier pipette tips between all manual liquid transfers.

10.4. During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.

10.5. Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.

10.6. Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.

10.7. Change gloves between samples and whenever contamination is suspected.

11.Keep reagent and reaction tubes capped or covered as much as possible.

12.Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold blocks at all times during preparation and use.

13. Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.

14. RNA should be maintained on cold blocks or on ice during preparation and use to ensure stability.

15.Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

ASSAY LIMITATIONS

The GeneStore Detection Expert 1ST © SARS-CoV-2 rRT-PCR kit performance was established using nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage samples only. Other specimen types have not been evaluated and should not be tested with this assay.

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.

Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

False-negative results may arise from:

- Improper sample collection
- Degradation of the SARS-CoV-2 RNA during shipping/storage
- Specimen collection after SARS-CoV-2 RNA can no longer be found in the specimen matrix
- Using unauthorized extraction or 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

Continued on next page.

ASSAY LIMITATIONS

- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The GeneStore Detection Expert 1S© SARS-CoV-2 rRT PCR Kit cannot rule out diseases caused by other bacterial or viral pathogens.

- 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.

Controls To Be Used With Detection Expert 1S © SARS CoV-2 rRT-PCR Kit

Patient samples must be collected according to appropriate laboratory guidelines. Positive and negative test controls must be included to accurately interpret patient test results.

Include the following controls:

Control	Used To Monitor	Assays
GeneStore Detection Expert © **SARS CoV-2 Positive Control Pack**	RT-PCR reaction setup and reagent integrity	Detection Expert 1S © SARS CoV-2 rRT PCR Assay
RNase P ** (Probe Mix Included in RT PCR assay)	Nucleic Acid Extraction Performance	Detection Expert 1S © SARS CoV-2 rRT PCR Assay
Negative Control (not supplied with kit, use nuclease free water)	Cross Contamination During RNA Extraction and reaction setup	Detection Expert 1S © SARS CoV-2 rRT PCR Assay

** The RNase P marker included in the multiplex SARS CoV2 RT PCR assay serves as a performance measure of the nucleic acid extraction method and an internal control. The RNaseP assay relies on the availability of the genomic DNA/RNA of the human sample that has been subject to extraction, and will amplify if the extraction performance is suitable.

2019-nCoV Positive Control Preparation:

Precautions: This reagent should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination. Freeze-thaw cycles should be avoided. Maintain on ice when thawed.

Make single use aliquots (approximately 30 µL) and store at ≤ -70°C.

Thaw a single aliquot of diluted positive control for each experiment and hold on ice until added to the plate. Discard any unused portion of the aliquot

Human Extraction Control (HEC) (RNaseP Assay)

The Human Extraction Control (HEC) is full-filed by the RNaseP assay that relies on the genomics RNA and DNA that is extracted from human sample being subjected to testing. The RNaseP marker is included in the RT PCR Assay for SARS CoV2, and uses the RNA template as the primary template.

No Template Control (NTC) (not provided)

1) Sterile, nuclease-free water

2) Aliquot in small volumes

3) Used to check for contamination during specimen extraction and/or plate set-up

GENERAL PREPARATION

Equipment Preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 10% bleach, 70% ethanol, to minimize the risk of nucleic acid contamination.

Nucleic Acid Extraction

Performance of the Real-Time RT-PCR assay is dependent upon the amount and quality of template RNA purified from human specimens. Utilise commercially available RNA extraction kits and procedures that have been qualified and validated for recovery and purity of viral RNA in nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage samples.

Manufacturer's recommended procedures are to be followed for sample extraction.

GUIDELINES FOR RT-PCR SETUP

IMPORTANT NOTE

• Prepare the run plate on ice and keep it on ice until it is loaded into the real-time PCR instrument.

• Run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.

• To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and samples, and always use aerosol barrier pipette tips.

• Maintain a RNase-free environment.

• Protect assays from light.

• Keep samples and components on ice during use.

• Include one positive control and one Negative Control in each run/plate, and setup and run the real-time PCR instrument.

RT PCR ASSAY SETUP

Note: Plate/run set-up configuration can vary with the number of specimens and workday organization. NTCs and positive controls must be included in each run.

1. If frozen, thaw the purified nucleic acid samples and reagents on ice.

2. Gently vortex the samples and reagents, then centrifuge briefly to collect liquid at the bottom of the 96-well plate/individual tubes.

Continued on next page.

GUIDELINES FOR RT-PCR SETUP

Preparing the cDNA Reaction Mix

For each run, combine the following components sufficient for the number of tests, plus one Positive and one Negative Control.

For Multiplex N1 and N2 Reaction		
Component	**Volume Per Sample or Control**	**Volume for N Samples + Controls**
ENYZME MIX	12.00 µL	12.00 * [N + 1] µL
PROBE MIX	4.50 µL	4.50 * [N + 1] µL
Total Reaction Mix Volume	16.50 µL	-

1. Dispense reagents into each respective labelled 1.5 mL microcentrifuge tube. After addition of the reagents, mix reaction mixtures by pipetting up and down. Do not vortex.

2. Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.

3. Set up reaction strip tubes or plates in a 96-well cooler rack.

GUIDELINES FOR RT-PCR SETUP

4. Dispense **16.50 µL** of each RT PCR preparation mix into the appropriate wells going across all the samples and controls.

5. Prior to moving to the nucleic acid handling area, prepare the No Template Control (NTC) reactions for column #1 in the assay preparation area.

6. Pipette **3.50 µL** of nuclease-free water into the NTC sample wells. Securely cap NTC wells before proceeding.

7. Cover the entire reaction plate/tubes and move the reaction plate/tubes to the specimen nucleic acid handling area.

Nucleic Acid Template Addition

8. Gently vortex nucleic acid sample tubes for approximately 5 seconds.

9. Centrifuge for 5 seconds to collect contents at the bottom of the tube.

10. After centrifugation, place extracted nucleic acid sample tubes in the cold rack.

11. Carefully pipette **3.50 µL** of the first sample into all the wells labelled for that sample (Tube 1: N1+N2 + RNaseP multiplex). Keep other sample wells covered during addition. Change tips after each addition.

12. Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking.

13. Change gloves often and when necessary to avoid contamination.

14. Repeat steps #11 and #12 for the remaining samples.

GUIDELINES FOR RT-PCR SETUP

Figure: Example of Reaction Master Mix Plate Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP	N1 N2 RP
B												
C												
D												
E												
F												
G												
H												

One Step RT PCR Cycling Conditions

Hold1: 42°C for 5 mins

Hold 2: 95°C for 5 mins

Cycling for 40 cycles as per conditions below:

Denaturation: 95°C for 15 seconds

Annealing: 58°C for 30 seconds

Acquisition Channel:

Multiplex Reaction:

- N1 is FAM (Green channel)
- N2 is HEX (Yellow channel)
- RNase P is CY5 (Red Channel)

Setting Up Your RT PCR Reaction Instrument

1. Close the PCR tubes. Make sure that the locking ring (accessory of the Rotor-Gene Instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run.

2. For the detection of SARS CoV 2 RNA, create a temperature profile according to the steps on the following page:

GUIDELINES FOR RT-PCR SETUP

Setting The General Assay Parameters

1. First, open the "New Run Wizard" dialog box. Check the "Locking Ring Attached" box and click "Next".

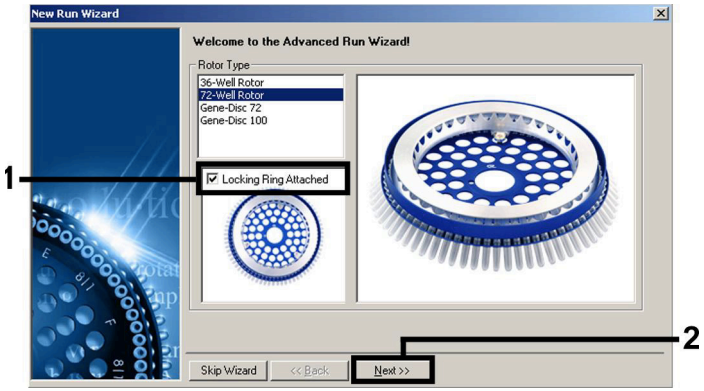


Figure. The "New Run Wizard" dialog box.

2. Select 25 for the PCR reaction volume and click "Next".

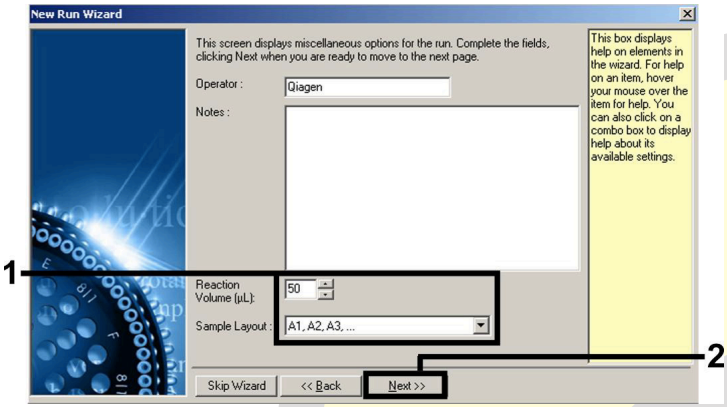


Figure. Setting the general assay parameters.

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GUIDELINES FOR RT-PCR SETUP

3. Click the “Edit Profile” button in the next “New Run Wizard” dialog box), and program the temperature profile as shown in the figures below.

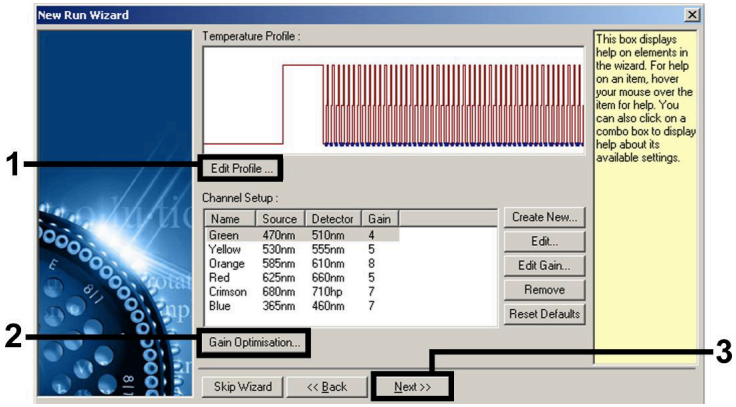

Figure. Editing the profile.

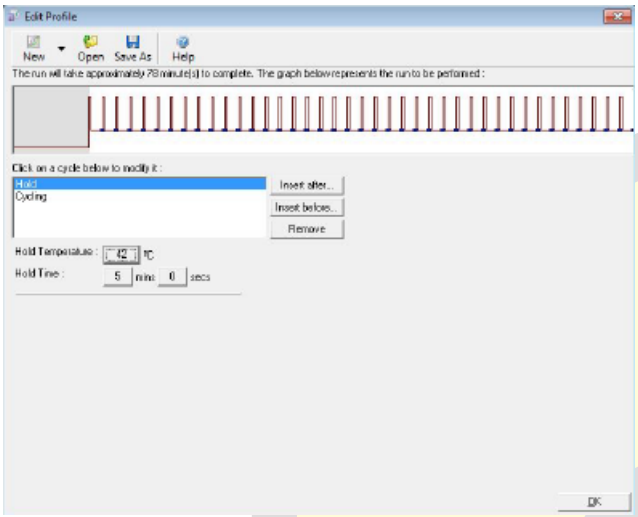

Figure. Reverse transcription of the RNA.

GUIDELINES FOR RT-PCR SETUP

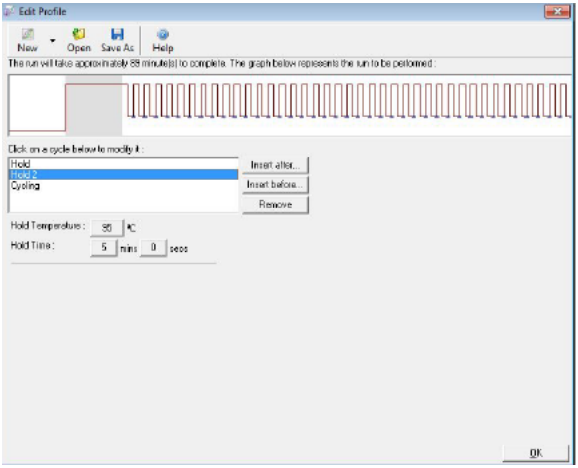

Figure. Initial activation of the hot-start enzyme.

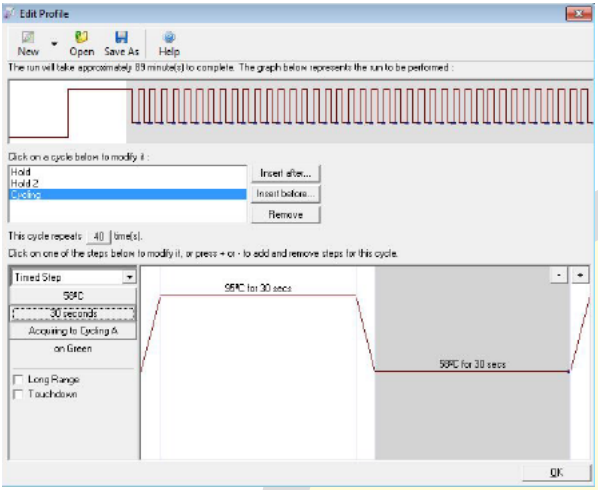

Figure. Amplification of the cDNA.

GUIDELINES FOR RT-PCR SETUP

3. The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click “Gain Optimisation” in the “New Run Wizard” dialog box to open the “Auto-Gain Optimisation Setup” dialog box. Set the calibration temperature to 58 to match the annealing temperature of the amplification program.

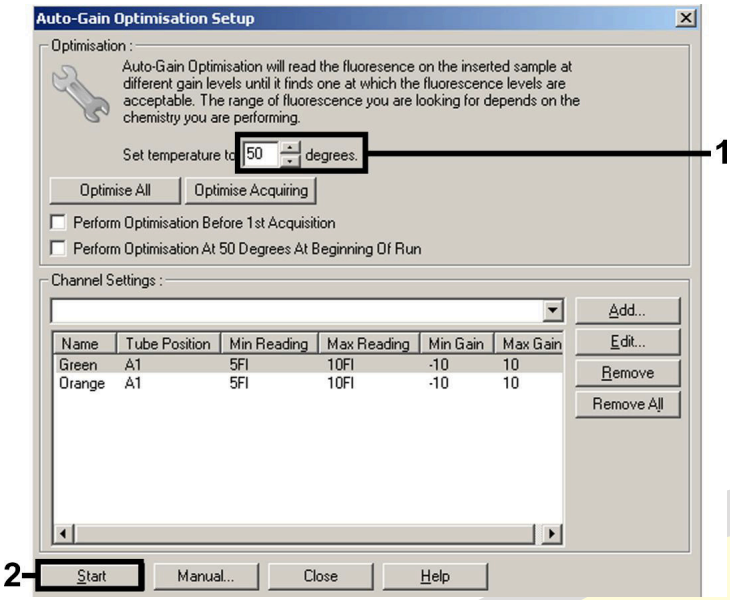

Figure. Adjusting the fluorescence channel sensitivity..

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GUIDELINES FOR RT-PCR SETUP

4. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure Click “Start Run”.

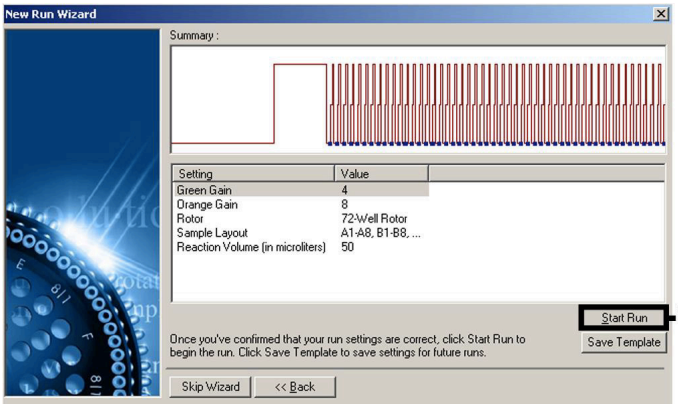


Figure. Starting the run.

5. After the run is finished, analyze the data.

5a. A signal is detected in fluorescence channel Cycling Green. The result of the analysis is positive: the sample contains SARS CoV 2 RNA.

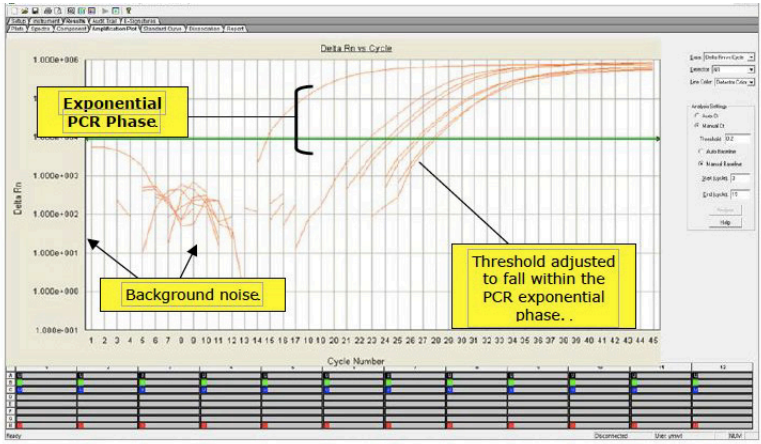
5b. In fluorescence channel Cycling Green no signal is detected. In the sample no SARS CoV 2 RNA is detectable. It can be considered negative.

- In the case of a negative SARS CoV 2 RT-PCR, the detected signal of the internal control rules out the possibility of RT-PCR inhibition.

DATA ANALYSIS

Note: Refer to your instrument manual for generating the amplification plot and generating Ct values for each sample.

Note on Determining Ct values: Adjust the threshold line until it lies within the exponential phase of the curves and above any background signal.



Determine the Ct value of each marker evaluated, and create a table as per the following format:

Sample ID	Marker N1 (Ct)	Marker N2 (Ct)	RNase P (Ct)
Sample			
Positive Control			
Negative Control			

RESULTS INTERPRETATION & REPORTING

Extraction and Positive Control Results and Interpretation

No Template Control (NTC) (Not Included in the Kit)

The NTC consists of using nuclease-free water in the rRT-PCR reactions instead of RNA. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and repeat the assay with strict adherence to the guidelines.

Positive Control

The positive control plasmids contain the sequence of the nucleocapsid gene regions 1 (N1) and 2 (N2) of SARS CoV2. The positive control will yield a positive result with the following primer and probe sets: N1 and N2 only.

Human Extraction Control (HEC) (RNaseP)

The RNase P functions as an HEC (see previous section on Assay Set Up), and is used as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. Successfully purified nucleic acid should yield a positive result with the RNaseP primer and probe set.

Continued on the next page.

RESULTS INTERPRETATION & REPORTING

Expected Performance of Controls Included in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel

Control Type	External Control Name	Used To Monitor	SARS CoV-2 Region 1	SARS CoV-2 Region 2	RNase P	Expected Ct Values
Positive	SARS CoV2 Positive Control	Substantial reagent failure including primer and probe integrity	[+]	[+]	[-]	< 35.00 Ct
Negative	NTC	Reagent and/or environmental contamination	[-]	[-]	[-]	None Detected
Extraction	RNaseP	Failure in lysis and extraction procedure, potential contamination during extraction	[-]	[-]	[+]	< 35.00 Ct

IMPORTANT NOTE

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

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RESULTS INTERPRETATION & REPORTING

RNaseP Extraction Control

1. All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within **35**.00 cycles (< **35**.00 Ct), thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:

- Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.

- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.

- Improper assay set up and execution.

- Reagent or equipment malfunction.

2. If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:

- If the N1 and N2 probes are positive even in the absence of a positive RP, the result should be considered valid. It is possible that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of SARS CoV 2 virus RNA in a clinical specimen.

- If all 2019-nCoV markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

RESULTS INTERPRETATION & REPORTING

SARS CoV 2 Markers: N1 and N2

1. When all controls exhibit the expected performance, a specimen is considered negative if all SARS-CoV-2 marker (N1, N2) cycle threshold growth curves DO NOT cross the threshold line within **35**.00 cycles (< **35**.00 Ct) AND the RNase P growth curve DOES cross the threshold line within **35**.00 cycles (< **35**.00 Ct).

2. When all controls exhibit the expected performance, a specimen is considered positive for 2019-nCoV if all 2019-nCoV marker (N1, N2) cycle threshold growth curves cross the threshold line within **35**.00 cycles (< **35**.00 Ct). The RNase P may or may not be positive as described above, but the 2019-nCoV result is still valid.

When all controls exhibit the expected performance and the growth curves for the 2019-nCoV markers (N1, N2) AND the RNase P marker DO NOT cross the cycle threshold growth curve within **35**.00 cycles (<**35**.00 Ct), the result is invalid. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.

4. When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2 but not both markers) crosses the threshold line within **35**.00 cycles (<**35**.00 Ct) the result is inconclusive. The extracted RNA should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the same result is obtained, report the inconclusive result. Consult with your state public health laboratory or CDC, as appropriate, to request guidance and/or to coordinate transfer of the specimen for additional analysis.

RESULT INTERPRETATION GUIDE

The table below lists the expected results for the GeneStore Detection Expert 1S© SARS-CoV-2 rRT PCR assay. If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please contact your GeneStore Representative for consultation and possible specimen referral.

SARS CoV-2 Region 1	SARS CoV-2 Region 2	RNase P	Result Interpretation	Report	Actions
[+]	[+]	[+/-]	SARS CoV 2 Detected	Positive SARS CoV 2	Report results to local health authority and sender.
If only one or both targets are positive		[+/-]	SARS CoV 2 Detected	Positive SARS CoV 2	Report results to local health authority and sender.
-	-	[+]	SARS CoV 2 Not Detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses.ii
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

i Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.
ii Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest that SARS CoV 2 infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If SARS CoV 2 infection is still suspected, re-testing should be considered in consultation with public health authorities.

QUALITY CONTROL

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to your governmental laboratory accreditation body.

- Quality control procedures are intended to monitor reagent and assay performance.

- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.

- Good laboratory practice (cGLP) recommends including a positive extraction control in each nucleic acid isolation batch.

- Always include a negative control (NTC), and the appropriate positive control (nCoVPC) in each amplification and detection run. All clinical samples should be tested for human RNAse P gene to control for specimen quality and extraction.

PERFORMANCE CHARACTERISTICS

Limit of Detection [LoD]

The LoD study established the lowest SARS-CoV-2 viral concentration (Genomic Copy Equivalents or GCE) that can be detected by the GeneStore Detection Expert 1S © SARS CoV-2 kit in a particular specimen type at least 95% of the time.

Banked Nasopharyngeal swab (NP) and Bronchoalveolar lavage (BAL) samples, obtained from patients in the years 2019, were pooled, respectively, and spiked with purified SARS-CoV-2 viral genomic RNA at several concentrations and processed through the GeneStore Detection Expert 1S© SARS CoV-2 kit workflow.

A three-phase approach was used to determine the LoD for each specimen type. In phases I and II, the preliminary LoD was established and confirmed in phase III by testing 20 replicates.

Continued on the next page.

PERFORMANCE CHARACTERISTICS

LoD Determination of Bronchoalveolar Lavage (BAL) Samples:

Effective Concentration	Replicate	Mean Ct		Interpretation	% Positive
		Region 1	Region 2		
100 GCE	1	34.03	33.02	Positive	100%
	2	33.90	33.25	Positive	
	3	34.10	33.10	Positive	
	4	34.04	33.10	Positive	
	5	34..10	33.20	Positive	
	6	34.10	33.03	Positive	
	7	34.15	33.02	Positive	
	8	34.12	33.05	Positive	
	9	31.10	33.06	Positive	
	10	34.09	33.07	Positive	
	11	34.20	33.06	Positive	
	12	34.05	33.06	Positive	
	13	34.02	33.15	Positive	
	14	34.06	33.10	Positive	
	15	34.25	33.12	Positive	
	16	34.11	33.12	Positive	
	17	34.11	33.18	Positive	
	18	34.25	33.20	Positive	
	19	34.06	33.22	Positive	
	20	34.03	33.10	Positive	

PERFORMANCE CHARACTERISTICS

LoD Determination of Nasopharyngeal Swab (NP) samples

Effective Concentration	Replicate	Mean Ct		Interpretation	% Positive
		Region 1	Region 2		
100 GCE	1	34.02	33.10	Positive	100%
	2	34.02	33.15	Positive	
	3	34.05	33.11	Positive	
	4	34.08	33.12	Positive	
	5	34.12	33.05	Positive	
	6	34.05	33.00	Positive	
	7	34.06	33.05	Positive	
	8	34.15	33.23	Positive	
	9	34.19	33.12	Positive	
	10	34.22	33.21	Positive	
	11	34.14	33.15	Positive	
	12	34.05	33.18	Positive	
	13	34.02	33.32	Positive	
	14	34.02	33.12	Positive	
	15	34.11	33.25	Positive	
	16	34.10	33.24	Positive	
	17	34.11	33.52	Positive	
	18	34.17	33.21	Positive	
	19	34.05	33.23	Positive	
	20	34.02	33.21	Positive	

PERFORMANCE CHARACTERISTICS

LOD Results

Specimen Type	Limit of Detection [GCE/Reaction]
Nasopharyngeal swab (NP)	100 GCE/Reaction
Bronchoalveolar lavage (BAL)	100 GCE/Reaction

Sensitivity Evaluation

The analytical sensitivity of the test will be further assessed by evaluating an US FDA-recommended reference material using an FDA developed protocol if applicable and/or when available.

In Silico Analysis of Primer and Probe Sequences

An alignment was performed with the oligonucleotide primer and probe sequences of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of February 1, 2020 to demonstrate the predicted inclusivity of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic panel.All the alignments show 100% identity of the CDC panel to the available 2019-nCoV sequences with the exception of one nucleotide mismatch with the N1 forward primer in one deposited sequence. The risk of a single mismatch resulting in a significant loss in reactivity, and false negative result, is low due to the design of the primers and probes with melting temperatures > 60°C and run conditions of the assay with annealing temperature at 58°C to tolerate one to two mismatches.

PERFORMANCE CHARACTERISTICS

Specificity/Exclusivity Testing: In Silico Analysis

BLASTn analysis queries of the 2019-nCoV rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on 10/03/2019; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

2019-nCoV_N1 Assay:
Probe sequence of 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probes, there are no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

2019-nCoV_N2 Assay:
The forward primer sequence of 2019-nCoV rRT-PCR assay N2 showed high sequence homology to Bat SARS-like coronaviruses. The reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probes, there is no prediction of potential false positive rRT-PCR results.

In summary, the 2019-nCoV rRT-PCR assay N1 and N2, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive rRT-PCR results.

PERFORMANCE CHARACTERISTICS

Clinical Evaluation

A clinical evaluation study was performed to evaluate the performance of the GeneStore Detection Expert 1S © SARS CoV-2 kit using nasopharyngeal swab (NP) and bronchoalveolar lavage (BAL) specimens.

A total of sixty (60) contrived positive specimens were tested:

- 30 contrived positive nasopharyngeal swab (NP) specimens
- 30 contrived positive bronchoalveolar lavage (BAL) specimens

Samples were contrived by spiking known concentrations of extracted SARS-CoV-2 viral genomic RNA, relative to the product LoD, into matrices which were determined to be negative by the GeneStore Detection Expert 1S © SARS CoV-2 kit prior to spiking in the RNA.

In addition to the contrived positive specimens, sixty (60) negative specimens were tested:

- 30 negative nasopharyngeal swab (NP) specimens
- 30 negative samples bronchoalveolar lavage (BAL) specimens
- All negative samples yielded negative results

Results for positive and negative samples are shown in the tables on the following page.

PERFORMANCE CHARACTERISTICS

Nasopharyngeal Swab (NP) Clinical Evaluation Study

Final RNA Concentration in Sample	Number of Positive Samples	Mean Ct	
		Region 1	Region 2
2X LoD	20/20	33.12	32.11
3X LoD	5/5	32.95	31.88
5X LoD	5/5	31.45	31.74

Bronchoalveolar lavage (BAL) Clinical Evaluation Study

Final RNA Concentration in Sample	Number of Positive Samples	Mean Ct	
		Region 1	Region 2
2X LoD	20/20	33.05	32.08
3X LoD	5/5	32.95	31.90
5X LoD	5/5	31.33	30.68

NOTES

NOTES

Bon Courage!

Good Luck !

TECHNICAL SUPPORT

For service and support information for this kit, email us:
corporate@genestore.eu

LIMITED WARRANTY

GeneStore France SAS and/or its affiliate(s) warrant their products as set forth in the GeneStore General Terms and Conditions of Sale at www.genestore.org/terms-and-conditions.html. If you have any questions, please contact us at corporate@genestore.eu.

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