SOLIS BIODYNE

DS-08-85 EN v2

SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit (IVD)

Qualitative Assay for Use on Real-Time PCR Instruments

Instructions for Use

08-85-00100	SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit	100 rxn
08-85-00400	SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit	400 rxn

Enzyme Mix 1

Enzyme Mix 2

Primer/Probe Mix

Positive Control

Nuclease-free Water

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1. Intended use

The SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit is an *in vitro* qualitative diagnostic test intended for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genetic material in biological samples obtained from the upper respiratory tract (nasopharyngeal swabs).

The product contains oligonucleotide primers and dual-labelled hydrolysis probes, as well as control material, for the use in real-time RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA.

The kit is intended for use exclusively by qualified and trained professionals in a diagnostic laboratory with the appropriate equipment and safety standards.

2. Summary and explanation of the test

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Symptoms of COVID-19 are variable including fever, headache, malaise, muscle pain, respiratory symptoms such as cough, dyspnoea, and hypoxemia.

The SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit is a molecular *in vitro* diagnostic test for the detection of SARS-CoV-2 RNA in biological specimens obtained from the upper respiratory tract (nasopharyngeal swabs).

Real-time RT-PCR technology utilizes reverse transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences and target-specific dual-labeled hydrolysis probes for the real-time detection of the amplified DNA.

The SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit is designed for simultaneous detection of three distinct regions in SARS-CoV-2 genome: Nucleocapsid gene (N, detected in FAM channel), Envelope gene (E, HEX channel) and RNA-dependent RNA polymerase gene (RdRP, ROX channel), as well as human RNase P transcript (RPP30, Cy5 channel). Human RNase P assay is designed to detect exclusively mRNA transcripts (genomic DNA is not amplified) and serves as an internal control used for monitoring over the processes of specimen collection and RNA extraction, RNA and PCR amplification, thereby reducing false negative results.

3. Description and content of the kit

Table 1. Components of the SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit

Product label	Main components	Amount, 100 reactions	Amount, 400 reactions	Cap colour
Enzyme Mix 1	HOT FIREPol® DNA polymerase, reaction buffer, dNTPs	1 × 440 µL	2 × 880 µL	Brown
Enzyme Mix 2	SOLIScript® Reverse Transcriptase, RiboGrip™ RNase Inhibitor	1 × 55 µL	1 × 220 µL	Yellow
Primer/Probe Mix	Primers and fluorescently labelled probes for SARS-CoV-2 regions in N (FAM), E (HEX), RdRP (ROX) genes, human RNase P mRNA (Cy5)	1 × 110 µL	1 × 440 µL	Blue
Positive Control	dsDNA Positive Control containing targets specific to the SARS-CoV-2 genomic regions N, E and RdRP targeted by the assay	1 × 80 µL	1 × 320 µL	Green
Nuclease-free Water	RNAse/DNAse free PCR grade water	1 × 1.25 mL	2 × 1.50 mL	Transparent

Fluorescence is emitted and individually recorded through optical measurements during the thermal cycling. The detection of the amplified fragment is performed by a fluorimeter using the channels shown **Table 2.**

Table 2. Fluorescence channel set-up

Target	Optical channel	Excitation	Emission
N gene	FAM	450-490 nm	510-530 nm
E gene	HEX/VIC/JOE	515-535 nm	560-580 nm
RdRP gene	Texas Red/ROX	560-590 nm	610-650 nm
RPP30 (Internal Control)	Cy5	620-650 nm	675-690 nm

4. Storage instructions

- Routine storage at -20°C until the expiration date indicated on the kit label.
- The kit can be stored at +4°C for up to one month.
- Freeze/thaw cycles should be minimized and not exceed 5 freeze/thaws.
- Primer/Probe Mix should be stored in the dark.

5. Additional necessary reagents and necessary equipment

The following list includes the items and materials that are required for use, but not included in the SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit.

- RNA extraction kit
- Real-Time PCR instrument (thermocycler)
- Centrifuge for 1.5 mL tubes and PCR-well strips or 96-well plate
- Appropriate PCR-well strips or 96-well plates as recommended by the manufacturer of the real-time PCR instrument
- Laboratory mixer, vortex or equivalent
- Single and multichannel adjustable micropipettes (1.00 µL to 1,000.0 µL)
- DNase-free RNase-free filter tips for micropipettes
- Powder-free disposable gloves and other personal protective equipment

The SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit has been tested and validated on the following Real-Time PCR equipment: Bio-Rad CFX96™ Real-Time PCR Detection System, Applied Biosystems™ 7500 Fast Real-Time PCR System, QuantStudio™ 5 Real-Time PCR System, LightCycler® 480 System, Rotor-Gene Q Instrument.

6. Precautions for users

- Biological samples should be always handled as potentially infectious material, direct contact with biological material should be avoided.
- Specimen processing should be performed in accordance with pertaining national biological safety regulations and following the recommended World Health Organization (WHO) guidelines on biosafety and biosecurity.
- Any work with the kit must be performed by qualified and trained professionals in a diagnostic laboratory with the appropriate equipment and safety standards.
- Do not mix reagents from different kits and/or lots and/or another supplier.
- Consult safety data sheets, upon request.
- Use dedicated rooms and workspaces for each step in the testing workflow: nucleic acid extraction, preparation of amplification reactions, amplification/detection of amplification products.
- Do not return samples, equipment, and reagents to the area in which the previous step was performed.

- Dedicated laboratory equipment, consumables, and personal protective equipment (lab coats, disposable gloves, eye protection etc.) should be used within each room.
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles, and a mask. Do not eat, drink, smoke or apply cosmetic products in the working area.
- Clean equipment and surfaces routinely with a fresh solution of 10% bleach (or equivalent decontamination agents that removes nucleic acids) followed by 70% ethanol after each use. Make sure that cleaning materials are compatible with the equipment used.
- SARS-CoV-2 Positive Control (N/E/RdRP) should be handled with caution to prevent possible contamination of other kit reagents and test RNA samples.
- Consult each Real-Time PCR instrument's reference manual for warnings, precautions, and procedures. Instruments must have been properly installed, calibrated, and maintained according to the manufacturer's recommendations.

7. Sampling and RNA extraction

Take necessary precautions during the collection, transport, storage, handling, and disposal of samples.

Clinical specimen processing should be performed in accordance with pertaining national biological safety regulations and following the recommended World Health Organization (WHO) guidelines on biosafety and biosecurity [1].

Performance of the SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit is dependent upon the amount and quality of RNA purified from human specimens. It is the responsibility of the user to validate conditions of collection, transport and storage of samples, and extraction of RNA by suitable systems to produce RNA of good quality.

The SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit performance was established using nasopharyngeal swab samples only. Other specimen types have not been evaluated and their compatibility with this assay should be tested and confirmed by the user. It is recommended to use extraction methods of RNA samples from respiratory specimens, and refer to manufacturer’s instructions of the extraction kit.

The following commercially available RNA extraction kits and procedures have been validated for recovery and purity of RNA for use with this assay:

- LifeRiver EX3600 (Shanghai ZJ Bio-Tech) with Ribonucleic Acid (RNA) Isolation Kit (Preloaded for Auto-Extraction) (Cat. No. ME-0014).
- QIAsymphony SP (Qiagen), QIAamp MinElute Virus Spin Kit (Cat. No. 57704).

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

8. Test workflow

8.1 Workspace preparation

- Use dedicated rooms and workspaces for each step in the testing workflow: nucleic acid extraction, preparation of amplification reactions, amplification/detection of amplification products.
- Dedicated laboratory equipment, consumables, and personal protective equipment (lab coats, disposable gloves, eye and face protection etc.) should be used within each room.
- Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used such as 10% bleach, 70% ethanol, and RNA/DNA removing agents to minimize the risk of nucleic acid contamination.

8.2 Preparation of the reaction mixture

IMPORTANT!

- All the work should be performed by qualified and trained professionals in a diagnostic laboratory with the appropriate equipment and safety standards.
- Positive Control should be handled with caution to prevent possible contamination of other kit reagents and test RNA samples.
- Use only reagents contained in this kit and reagents recommended by the manufacturer.
- Do not combine or mix reagents from different lots.
- Do not combine reagents from kits from different manufacturers.
- Best use RNA immediately after extraction and keep it on ice.
- Alternatively, use RNA stored at -20°C to -80°C and avoid prolonged exposure to room temperature, thaw on ice and immediately refreeze the RNA, if needed.

PREPARE THE REACTIONS

Determine the total number of required reactions including test samples and controls. In addition to test RNA samples, each assay should include:

- One Positive Control (PC) using the Positive Control (N/E/RdRP) provided in the kit, as template.
- One Negative Control (NC) using Nuclease-free Water provided in the kit, as template.

Adding an additional 10% volume to the reaction master mix is recommended to compensate for pipetting errors.

1. All kit reagents and test samples should be thawed completely on ice, mixed (by inverting, pipetting or gentle vortexing) and centrifuged briefly before use.
2. Prepare a reaction master mix on ice. To avoid the pipetting errors due to viscosity of the buffer, enzyme mixes should be pipetted carefully and slowly. To prepare the reaction master mix, the individual components of the kit must be mixed following the protocol below.

Reaction mixture setup

Table 3. Reaction mixture components and setup

Reaction mix component	Volume per Sample or Control	Volume for n Samples plus 2 Controls [1]	Volume for 94 Samples plus 2 Controls [1]
Nuclease-free Water	6.5 µL	7.15 x (n + 2) µL	686.4 µL
Primer/Probe Mix	1.0 µL	1.10 x (n + 2) µL	105.6 µL
Enzyme Mix 1	4.0 µL	4.40 x (n + 2) µL	422.4 µL
Enzyme Mix 2	0.5 µL	0.55 x (n + 2) µL	52.8 µL
Total reaction master mix volume	12 µL	-	1267.2 µL

[1] All volumes include 10% overage for pipette error.

3. Mix the reaction mix thoroughly, centrifuge briefly and distribute 12 µl of the reaction master mix prepared in step 2 to each reaction well according to the plate setup.
4. Add 8 µl of test RNA sample, Negative Control and Positive Control to the appropriate wells according to the plate setup.
5. Seal the plate with an appropriate seal, centrifuge reactions briefly, and place in the Real-Time PCR instrument.

8.3 PCR instrument set-up and detection channel setting

Consult Real-Time PCR instrument's manual for warnings, precautions, and procedures. The instrument must have been properly installed, calibrated, and maintained according to the manufacturer's recommendations.

The detection of amplified fragments is performed in the following channels:

- N gene: FAM channel
- E gene: VIC/HEX/JOE channel
- RdRP gene: Cal Red 610/ROX/Texas Red channel
- Internal Control RNase P, RPP30: Quasar 670/Cy5 channel

Setting up the Bio-Rad CFX96 Instrument:

1. Open the Bio-Rad CFX Maestro software, click ‘File’, select ´New´ then ´Protocol´ to open the Protocol Editor.
2. Enter a Sample Volume of 20 ul.
3. Enter the Thermal Cycling Protocol as in Table 4. Confirm to have the plate read after the annealing/extension step (indicated by the camera symbol in the Protocol Editor graphics).
4. Save the protocol, click ´File´ and ´Save as´.

Table 4. Thermal cycling protocol

Step	Temperature	Time	Cycles
Reverse transcription	50°C	10 minutes	1
Initial activation	95°C	10 minutes	1
Denaturation	95°C	3 seconds	45
Annealing/extension	62°C	15 seconds*	

*Acquisition must be performed at the end of this stage

5. Plate setting: click ´File´, select ´New´ then ´Plate´ to open the Plate Editor. From ´Settings´ menu choose the appropriate Plate Size and Plate Type. In the Plate Editor window choose Scan Mode as ´All Channels´. From the ´Select Fluorophores´ menu select FAM, HEX, Texas Red, and Cy5. Define the Sample Type, Target Names, and plate setup as per your experiment plan.
6. Save the plate setting, click ´File´ and ´Save as´.
7. Start the run: click ´Run´, select ´User-defined Run´ then follow through the prompts, choose the correct Protocol and Plate setup options. Click ´Start Run´.

Setting up the Applied Biosystem 7500 Fast Instrument:

1. Open 7500 Software, create a new experiment selecting ‘Design Wizard’. Define the experiment properties, add ‘Experiment Name’, choose ‘7500 (96 wells), ‘Quantitation’ for experiment type, then click ‘Next’.
2. Plate settings: select method ‘Standard Curve’, ‘TaqMan Reagents’ for the reagents, ‘Standard’ for ramp speed, ‘RNA’ for template type, then click ‘Next’. Set up targets number to ‘4’. Click the ‘Enter Target Name’ and set ‘Reporter’ to FAM, VIC, ROX, Cy5 and ‘Quencher’ to ‘NFQ-MGB’. Click ‘Next’. Set up samples: the number of rows, replicates, and negative controls. Select ‘All Samples/Target Reactions’. Select the dye to use as the passive reference ‘None’ and then click ‘Next’.
3. Program settings: click the ‘Reaction Volume Per Well’ field, then enter 20 ul and set the program as below. Click ‘Save Run Method’.

Table 5. Thermal cycling protocol

Step	Temperature	Time	Cycles
Reverse transcription	50°C	10 minutes	1
Initial activation	95°C	10 minutes	1
Denaturation	95°C	10 seconds	45
Annealing/extension	62°C	30 seconds*	

*Data acquisition mode

4. Start the run: in home screen click on ‘Run’, open the run method file. Push the tray door to open it, load the plate, close the tray door. Click on ‘Start Run’ to start the experiment.

Setting up the QuantStudio 5 Instrument:

1. Open QuantStudio™ Design and Analysis Software and click on ‘Create New Experiment’. Add to ‘Properties’ file name, select ‘QuantStudio 5’ for instrument type, ‘TaqMan Reagents’ for chemistry and ‘Standard’ for run mode.
2. Program settings: in ‘Method’ tab, set reaction volume to 20 ul and the program as below.

Table 6. Thermal cycling protocol

Step	Temperature	Time	Cycles
Reverse transcription	50°C	10 minutes	1
Initial activation	95°C	10 minutes	1
Denaturation	95°C	10 seconds	45
Annealing/extension	62°C	30 seconds*	

*Data acquisition mode

3. Plate settings: in ‘Plate’ tab, select ‘Passive Reference’ as ‘None’. Choose advanced setup and assign targets and samples. Add four targets and set ‘Reporter’ to FAM, VIC, ROX, Cy5 and ‘Quencher’ to ‘NFQ-MGB’. Set samples and click ‘Next’.
4. Start the run: click to open lid then put in the samples, click to close the lid. Click ‘START RUN’ and choose instrument number in opening sub-icon.

Setting up the LightCycler480 Instrument

1. Open LightCycler 480 software, select ‘New Experiment’.
2. Program settings: set the detection mode to ‘Multi Color Hydrolysis Probe/UPL Probe’ and the detection channel to FAM, VIC, ROX and Cy5. Add RT, Inactivation and cycling steps in program table. Select ‘Amplification Temperature Targets’ and add denaturation and annealing steps. The amplification parameters are as following. Click ‘Subset Editor’, select wells, and click ‘Apply’.

Table 7. Thermal cycling protocol

Step	Temperature	Time	Cycles
Reverse transcription	50°C	10 minutes	1
Initial activation	95°C	10 minutes	1
Denaturation	95°C	10 seconds	45
Annealing/extension	62°C	30 seconds*	

*Data acquisition mode

3. Select ‘Sample Editor’ to set the sample name, save the file. Click to open lid then put in the samples, click to close the lid. Run the program after setting.

Setting of Rotor-Gene Q Instrument

1. Open Rotor-Gene Q software, click at ‘Empty Run’ and choose ‘New’. Apply rotor type and click ‘Next’.

2. Program settings: set 'Reaction volume' to 20 ul and click 'Next'. Choose 'Edit Profile' and click 'Insert after…'. Add two 'New Hold at Temperature' and one 'New Cycling' steps. Click on hold and cycling steps to specify temperature, time, and repeats. In cycling, firstly click at the Annealing/extension step, then click at 'Not Acquiring'. Add FAM, VIC, ROX and Cy5 (green, yellow, orange, red respectively) from dye channel selection chart. Click 'OK', twice. Choose 'Gain Optimization'. Click 'Channel Settings' adding green, yellow, orange, and red step by step. Mark 'Perform Optimization Before First Acquisition' as chosen and 'Close' the page.

Table 8. Thermal cycling protocol

Step	Temperature	Time	Cycles
Reverse transcription	50°C	10 minutes	1
Initial activation	95°C	10 minutes	1
Denaturation	95°C	3 seconds	45
Annealing/extension	62°C	15 seconds*	

*Data acquisition mode

3. Start the run: click 'Next' and 'Save Template' and 'Start Run'.

8.4 Quality control and sample interpretation

Follow the Real-Time PCR equipment manufacturer's manual and your internal laboratory and testing procedures when setting the analysis conditions, number and type of samples, plate setup and distribution of sample, as well as the type of plasticware used (strips, tubes, plates).

The specific software for the Real-Time PCR instrument employed must be used to analyse amplification results.

Assessment of clinical RNA samples test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. One Negative Control (NC) and one Positive Control (PC) should be processed with each run.

Testing results are considered valid if the following criteria are satisfied:

- Negative Control (NC) shows no amplification (i.e. Cq values > 40.0) in any fluorescence channel (FAM, HEX, ROX, Cy5 channels).
- Positive Control (PC) produces positive results (Cq values < 38.0) in all channels (FAM, N gene; HEX, E gene; ROX, RdRP gene).

NOTE: quantification cycle (Cq) = cycle threshold (Ct) = crossing point (Cp).

If quality control criteria are not satisfied, sample retesting should be considered. Sample retesting needs to be performed by re-testing of nucleic acid and/or extraction of RNA from the specimen and repeating RT-qPCR test. If the retesting result remains inconclusive, collection and testing of a new clinical sample should be considered.

Please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification. Testing results should be inspected both in logarithmic and linear scale

view and compared with the negative control (NC). If necessary, the threshold should be adjusted.

All the results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms, and other diagnostic tests.

- The signal for any SARS-CoV-2 target (N gene, E gene, RdRP gene) is considered positive (POS) if the amplification curve crosses the threshold line within 40 cycles (Cq < 40).
- The Rnase P (RPP30, Cy5, internal control) shows (POS) or not (NEG) an amplification signal (Cq ≤ 40 or no signal). Detection of Internal Control (Rnase P, RPP30, detected in Cy5 channel) may not be necessary because a high copy number of SARS-CoV-2 material (N, E, RdRP targets) causing preferential amplification of target-specific fragments.

For interpretation of patient sample results, use the below table.

Table 9. Assay results interpretation guideline

N (FAM)	E (HEX)	RdRP (ROX)	Rnase P, RPP30 (Cy5)	Result	Action
One SARS-CoV-2 target is POS			POS or NEG	SARS-CoV-2 RNA Inconclusive	Repeat test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.[1]
Two or more SARS-CoV-2 targets are POS			POS or NEG	SARS-CoV-2 RNA Detected	Report results should be provided to healthcare provider.
NEG	NEG	NEG	POS	SARS-CoV-2 RNA Not Detected	Results should be reported to healthcare provider. Testing for other respiratory pathogens should be considered.
NEG	NEG	NEG	NEG	NA	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.[1]

[1] Sample retesting needs to be performed by re-testing of nucleic acid and/or extraction of RNA from the specimen and repeating RT-qPCR test. If the retesting result remains inconclusive, collection and testing of a new clinical sample should be considered.

9. Limitations of the method

- The SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit performance was established using nasopharyngeal swab samples only. Other specimen types have not been evaluated and their compatibility with this assay should be tested and confirmed by the user.
- Clinical samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of clinical specimens may affect the ability of the assay to detect the target sequences.
- Extraction of nucleic acid from clinical samples must be performed according to the specified methods listed in the instructions of the nucleic extraction protocol. It is the responsibility of the user to validate suitable extraction approaches and processing systems to produce RNA of good quality.
- The procedures in this manual must be followed as described. Any deviations may result in assay failure or cause erroneous results.
- Good laboratory practice is required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded as standard laboratory waste.
- Mutations within the target sequence of SARS-CoV-2 may affect the SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit primer and/or probe binding. Detection rate and performance at the time of testing may vary depending on the circulating SARS-CoV-2 variants, including newly emerging strains of SARS-CoV-2 and their prevalence.
- False negative results may be caused by:
  - Unsuitable collection, handling and/or storage of samples.
  - Use of non-validated extraction kit or PCR platform, failure to follow procedures and instructions described in the manuals of nucleic extraction kits and PCR platforms.
  - Failure to follow procedures in this manual.
- False positive results may be caused by:
  - Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
  - Unsuitable handling of amplified product resulting in a contamination issue.
- All results should be interpreted by a respectively trained health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.

10. Performance characteristics

10.1 Analytical sensitivity or Limit of detection (LoD)

The limit of detection (LoD) is defined as the lowest concentration of the analyte that can be reliably detected. The LoD refers to the limit associated with 95% probability of obtaining a correct testing result.

Evaluation of analytical sensitivity (Limit of Detection, LoD) was performed using mock positive SARS-CoV-2 RNA samples containing the Helix Elite™ Synthetic Standard SARS-CoV-2

Synthetic RNA Pool (N/E/RdRp/S Gene Targets) (Microbiologics, Cat. No. HE0061S) and Quantitative PCR Human Reference Total RNA (Agilent, Cat. No. 750500). Dilutions of the Helix Elite™ Synthetic Standard SARS-CoV-2 Synthetic RNA Pool were prepared by serial dilutions of the resuspended stock standard, resulting in reactions with 10 000, 1 000, 100, 10, 5 copies of synthetic SARS-CoV-2 template per reaction. Each reaction contained 0.6 ng of Quantitative PCR Human Reference Total RNA (Agilent, Cat. No. 750500). The preliminary LoD assay was performed with three replicates for each prepared dilution. Samples were tested on Bio-Rad CFX96™ Real-Time PCR Detection System.

LoD assessment established reliable synthetic SARS-CoV-2 ssRNA template detection of all the targets at 10 copies per reaction. **Table 10** summarizes the results of 24 technical replicates at determined assay LoD. This data demonstrates that the SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit detects 10 copies/reaction of SARS-CoV-2 synthetic ssRNA with ≥ 95% probability.

Table 10. LoD assessment results of SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit at Synthetic Standard SARS-CoV-2 ssRNA concentration of 10 copies per reaction.

Target	N gene	E gene	RdRP gene
Positive calls/Total number of samples	24/24	24/24	22/24
Detection success	100%	100%	91.6%

10.2 Analytical specificity

In silico assessment of the SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit primer and probe sequences for cross-reactivity against the genomes of SARS-CoV-2 near-neighbour species/strains and organisms was performed. A total of 42 organisms representing genomes of different pathogens (bacteria, viruses, and fungi) was analysed. Sequence data was downloaded from the NCBI Nucleotide collection (NCBI GenBank, https://www.ncbi.nlm.nih.gov/nucleotide) using Entrez Direct (EDirect) utility. The search was limited to using the taxonomy ID (txid) of the respective pathogen.

The results of the analysis showed that tested 42 species/organisms/strains showed less than 80% BLAST homology of the sequences of primers and probes for all the individual assays (N, E, RdRP genes). Detailed individual and combinatorial analysis of sequence alignments of SARS-CoV-2 assay primers and probes showed that there is no potential unintended cross-reactivity. Results are summarized in **Table 11**.

Table 11. *In silico* cross-reactivity analysis.

Pathogen	NCBI Taxonomy ID	Total n of genomes analysed
Adenovirus	NCBI:txid10509	1049
Bacillus anthracosis	NCBI:txid1392	927
Bordetella pertussis	NCBI:txid520	2782
Candida albicans	NCBI:txid5476	870
Chlamydia pneumoniae	NCBI:txid83558	375
Chlamydia psittaci	NCBI:txid83554	145
Corynebacterium diphtheriae	NCBI:txid1717	243
Coxiella burnetii	NCBI:txid777	178
Enterovirus A-D	NCBI:txid138948, NCBI:txid138949, NCBI:txid138950, NCBI:txid138951	3963
Haemophilus influenzae	NCBI:txid727	473
Human coronavirus 229E	NCBI:txid11137	72
Human coronavirus HKU1	NCBI:txid290028	39
Human coronavirus NL63	NCBI:txid277944	60
Human coronavirus OC43	NCBI:txid31631	182
Human mastadenovirus C	NCBI:txid129951	101
Human Metapneumovirus	NCBI:txid162145	157
Human Parainfluenza virus 1-4	NCBI:txid12730, NCBI:txid11216, NCBI:txid1979160, NCBI:txid1979161	482
Human Respiratory syncytial virus	NCBI:txid11250	2045
Influenza A virus	NCBI:txid11320	3764
Influenza B virus	NCBI:txid11520	120
Influenza C virus	NCBI:txid11552	13
Legionella longbeachae	NCBI:txid450	31
Legionella non-pneumophila	NCBI:txid445,	255

	exclude NCBI:txid446	
Legionella pneumophila	NCBI:txid446	983
Leptospira	NCBI:txid171	690
MERS-coronavirus	NCBI:txid1335626	612
Moraxella catarrhalis	NCBI:txid480	108
Moraxella osloensis	NCBI:txid34062	110
Mycobacterium tuberculosis	NCBI:txid1773	4136
Mycoplasma pneumoniae	NCBI:txid2104	159
Neisseria elongata	NCBI:txid495	11
Neisseria meningitidis	NCBI:txid487	1414
Parechovirus	NCBI:txid138954	200
Pneumocystis jirovecii	NCBI:txid42068	16
Pseudomonas aeruginosa	NCBI:txid287	14576
Rhinovirus	NCBI:txid147711, NCBI:txid147712, NCBI:txid463676	362
SARS-coronavirus	NCBI:txid694009, exclude HCoV-19 (taxid:2697049)	331
Staphylococcus aureus	NCBI:txid1280	23552
Staphylococcus epidermidis	NCBI:txid1282	2621
Streptococcus pneumoniae	NCBI:txid1313	50542
Streptococcus pyogenes	NCBI:txid1314	2888
Tatlockia micdadei	NCBI:txid451	5

10.3 Inclusivity or analytical reactivity

Global inclusivity was assessed using *in silico* analysis of all SARS-CoV-2 genome sequences available at the time of the analysis in the NCBI Nucleotide collection (NCBI GenBank, https://www.ncbi.nlm.nih.gov/nucleotide) (as of 19 November 2021).

A total of 2 253 291 SARS-CoV-2 genome sequences were downloaded from the NCBI GenBank. The SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit primer/probe sequences targeting SARS-CoV-2 genome regions encoding N gene, E gene, and RdRP gene were mapped to the SARS-CoV-2 sequences to check for potential amplicon producing matches. For each individual gene assay, criteria for the positive result were as follows: (i) all

three oligonucleotides of each individual gene assay (probe + both primers) give 100% identity (homology) and a full-length match, (ii) the probe is located between the two primers, and (iii) the product length is less than 1000 bp. *In silico* analysis showed 100% primer and probe sequence identity with more than 98% of SARS-CoV-2 genomes (99.07% for the N gene assay, 99.02% for the E gene assay, 98.22% for the RdRP gene assay) deposited in the NCBI GenBank database.

***In silico* assessment of the molecular assay for detection of circulating SARS-CoV-2 variants of potential public health importance**

SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit primer and probe sequences of N, E, and RdRP genes were aligned to the genome sequences of SARS-CoV-2 Variants of Concern (VOC) and Variants of Interest (VOI) (as of November 12,2021) [2]. Sequences were downloaded from the NCBI Nucleotide collection (NCBI GenBank, https://www.ncbi.nlm.nih.gov/nucleotide) on November 19, 2021. *In silico* analysis of the SARS-CoV-2 primer and probe sets was performed against 12 different SARS-CoV-2 lineages: B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), B.1.427+B.1.429 (Epsilon), B.1.525 (Eta), B.1.526 (Iota), B.1.617.1 (Kappa), P.2 (Zeta), B.1.621 (Mu), C.37 (Lambda), P.3 (Theta). Results are summarized in **Table 12**.

In silico analysis predicts that assay should detect currently circulating SARS-CoV-2 lineages as it shows 100% primer and probe sequence identity with more than 97% of SARS-CoV-2 genomes of abovementioned lineages deposited in the NCBI GenBank database. The analysis of P.2 (Zeta) lineage showed that RdRP gene assay can detect 91.50% of the SARS-CoV-2 sequences identified and designated as P.2 (Zeta) lineage. The performance with P.2 (Zeta) lineage is not expected to be impacted as the detection of two out of three assay targets results in a positive evaluation.

Table 12. Results of the *in silico* analysis of VOC and VOI detection.

	RdRP assay	E assay	N assay
B.1.1.7 (Alpha)			
total n of genomes identified (analysed)	495858 (495800)	495858 (495801)	495858 (495776)
detection rate (% of analysed genomes)	99.69	99.50	99.94
B.1.351 (Beta)			
total n of genomes identified (analysed)	5121 (5119)	5121 (5121)	5121 (5121)
detection rate (% of analysed genomes)	98.26	99.45	98.87
P.1 (Gamma)			
total n of genomes identified (analysed)	13617 (13598)	13617 (13615)	13617 (13616)
detection rate (% of analysed genomes)	99.71	99.32	99.29
B.1.617.2 (Delta)			
total n of genomes identified (analysed)	98188 (98153)	98188 (98174)	98188 (98148)
detection rate (% of analysed genomes)	98.86	99.11	99.20

B.1.427+B.1.429 (Epsilon)			
B.1.427			
total n of genomes identified (analysed)	10099 (10096)	10099 (10097)	10099 (10096)
detection rate (% of analysed genomes)	95.38	99.38	97.72
B.1.429			
total n of genomes identified (analysed)	23628 (23625)	23628 (23622)	23628 (23624)
detection rate (% of analysed genomes)	97.36	98.29	99.54
B.1.525 (Eta)			
total n of genomes identified (analysed)	2221 (2221)	2221 (2221)	2221 (2220)
detection rate (% of analysed genomes)	98.15	97.39	99.23
B.1.526 (Iota)			
total n of genomes identified (analysed)	32291 (32281)	32291 (32289)	32291 (32279)
detection rate (% of analysed genomes)	97.37	99.29	98.44
B.1.617.1 (Kappa)			
total n of genomes identified (analysed)	822 (822)	822 (822)	822 (822)
detection rate (% of analysed genomes)	99.15	100.00	99.76
P.2 (Zeta)			
total n of genomes identified (analysed)	1060 (1059)	1060 (1060)	1060 (1060)
detection rate (% of analysed genomes)	91.50	99.62	97.64
B.1.621 (Mu)			
total n of genomes identified (analysed)	3561 (3555)	3561 (3556)	3561 (3560)
detection rate (% of analysed genomes)	99.94	98.31	98.93
C.37 (Lambda)			
total n of genomes identified (analysed)	1054 (1054)	1054 (1054)	1054 (1054)
detection rate (% of analysed genomes)	99.24	99.15	98.96
P.3 (Theta)			
total n of genomes identified (analysed)	40 (40)	40 (40)	40 (40)
detection rate (% of analysed genomes)	100.00	100.00	100.00

In silico **and experimental assessment of the molecular assay for detection of the SARS-CoV-2 Omicron variant**

SARS-CoV-2 assay RdRP, E and N gene primer and probe sequences were aligned to the GISAID sequence EPI_ISL_6841980 representing the origin of the synthetic ssRNA reference viral control material produced by Twist Bioscience [(Control 48 (B.1.1.529/BA.1) Hong Kong/HKU-211129-001/2021, catalog #105204)].

Alignment of the RdRP, E, and N gene primer and probe sequences to the EPI_ISL_6841980 sequence revealed two single nucleotide mismatches, one located in the sequence of the forward primer of the E gene (5th nucleotide from the 5' end) and the other in the sequence of the N gene probe (3rd nucleotide from the 5' end).

Evaluation of molecular assay components that did not match 100% to the target sequence EPI_ISL_6841980 combined with mismatch melting temperature analysis indicated that E gene forward primer and N gene probe mismatches are unlikely to impact assay function. The E gene forward primer and N gene probe exhibit melting temperatures from the template strand that are higher than the annealing temperature for the qPCR (E gene primer in the assay Tm 64.9°C, E gene primer with 100% homology to EPI_ISL_6841980 Tm 63.2°C; N gene probe in the assay Tm 73.5°C, N gene probe with 100% homology to EPI_ISL_6841980 Tm 71.7°C).

SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit molecular assay sensitivity test was performed with four 10-fold serial dilutions (10 000, 1 000, 100, 10 copies/reaction) of the Twist Bioscience Control 48 (B.1.1.529/BA.1) consisting of six non-overlapping 5 kb fragments generated from Twist Gene Fragments then transcribed into ssRNA. Based on the product description, Twist Bioscience synthetic viral controls provide coverage of greater than 99.9% of the bases of the SARS-CoV-2 viral genome.

Experimental results confirmed that SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit molecular assay sensitivity with SARS-CoV-2 synthetic viral control for Omicron variant is not affected by single nucleotide mismatches in the E gene forward primer and N gene probe sequences.

Primers and probes used in SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit and the amplified regions were also aligned to Omicron BA.2, BA.4 and BA.5 sequences.

Complete genomes of the isolates of given lineages that have high sequence coverage were downloaded from GISAID (https://www.gisaid.org/) and a minimum of 100 and a maximum of 1000 sequences were used for the analysis of each strain, depending on the number of available sequences. All the isolates of either BA.2, BA.4 or BA.5 were mapped to Wuhan-Hu-1 reference strain (GenBank: MN908947). From these assemblies, a consensus sequence for each strain was extracted. Subsequently, the obtained consensus sequences of BA.2, BA.4 and BA.5 strains were aligned with the experimentally tested SARS-CoV-2 strains (Wuhan-Hu-1 and BA.1) and the amplicon regions of SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit were analysed.

If no additional nucleotide changes within the amplicon regions are detected between the experimentally tested strains (Wuhan-Hu-1 and BA.1) and the analysis strain, the kit is deemed functional.

As the analysed Omicron variants BA.2, BA.4 and BA.5 share 100% sequence identity with the experimentally tested SARS-CoV-2 Omicron variant BA.1 within the amplicon regions of

the kit, we can conclude the SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit to be functional with Omicron variants BA.2, BA.4 and BA.5.

10.4 Clinical sensitivity and specificity

The clinical performance of SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit was evaluated. The evaluation was performed on 45 confirmed positive and 24 individual negative clinical samples. The RNA extraction from nasopharyngeal swab samples was performed using LifeRiver EX3600 (Shanghai ZJ Bio-Tech) with Ribonucleic Acid (RNA) Isolation Kit (Preloaded for Auto-Extraction) (ME-0014). Samples were tested on Bio-Rad CFX96™ Real-Time PCR Detection System and results were compared to the results obtained by a molecular comparator assay. Percent positive agreement (PPA) and negative percent agreement with 95% confidence interval was calculated. Clinical performance assay results are illustrated in **Table 13**.

Table 13. Results of the clinical evaluation study of SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit.

		Reference detection assay (CE-IVD)		
		Positive	**Negative**	**Total**
SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit	**Positive**	45	0	45
	Negative	0	24	24
	Total	45	24	69

Performance against the results obtained with the reference molecular detection methods are:
Positive Percent Agreement 45/45 = 100% (95% CI: 92.1% - 100%)
Negative Percent Agreement 24/24 = 100% (95% CI: 85.8%-100%)

11. Disposal

Dispose of unused kit reagents, human clinical samples, and sealed amplification plates as laboratory clinical waste according to local, state, and federal regulations.

12. Version history

Instruction for Use version DS-08-85 EN v2, July 15, 2022.

Version 2 has updates on:

Chapter 5 ´Additional necessary reagents and necessary equipment´ on page 5. List of tested and validated Real-Time PCR equipment was updated.

Chapter 8.3 ´PCR instrument set-up and detection channel setting´ on pages 8 to 11.

Chapter 10.3 Functionality with Omicron variants BA.2, BA.4 and BA.5

Chapter 17 ´References´ on page 22

Table numbering was updated throughout the document.

13. Solis BioDyne Quality Control

Manufactured by Solis BioDyne, in compliance with the ISO 9001 and ISO 13485 certified Quality Management System.

In accordance with Solis BioDyne ISO 13485 certified Quality Management System and to ensure consistent product quality, each batch of the SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit is tested against predetermined quality specifications.

14. Technical support

For Technical support, please contact our dedicated technical support team on:

Phone: +372 740 9960

Email: support@solisbiodyne.com

15. Trademarks and Disclaimers

SOLIScript®, HOT FIREPol® are EU registered trademarks of Solis BioDyne OÜ. RiboGrip™ is a trademark of Solis BioDyne OÜ.

All other trademarks that appear in this IFU are the property of their respective owners.

16. Explanation of symbols

Explanation of symbols

	Batch code		Use by
	Catalogue number		Manufactured by
	Contains sufficient for <n> tests		Store at
	This product fulfils the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices		For *in vitro* diagnostic use

17. References

[1] Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs). (https://www.who.int/publications/i/item/9789240011311)

[2] https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/, https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html)