SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit 2.0

Catalogue Number	Size (20 µl reactions)
08-83-00100	100 reactions
08-83-00250	250 reactions

Shipping:

On blue ice or at 2°C to 8°C

Batch Number and Expiry Date:

See vial

Storage and Stability:

- Routine storage at –20°C until Expiry Date
- The kit can be stored at +4°C for up to 2 month

Reaction setup:

At room temperature

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

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Product description:

- SOLIScript® SARS-CoV-2 RT-qPCR Multiplex Assay Kit is a multiplex RT-qPCR assay for the qualitative detection of SARS-CoV-2 viral RNA plus a human RNase P assay to assess sample adequacy.
- The extraction and purification of nucleic acids should be performed using commercially available extraction kits or automated nucleic acid extraction platforms.
- 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.
- The kit includes the proprietary in-silico designed SOLIScript® Reverse Transcriptase characterized by enhanced ability to tolerate higher temperature (up to 65ºC). It also contains an RNase Inhibitor RiboGrip™ to protect RNA samples from degradation. HOT FIREPol® DNA polymerase is a chemically blocked hot-start polymerase activated by a 10 min incubation step at 95°C.

Kit components:

Kit component	Size	
	100 rxn	250 rxn
40x One-step SOLIScript® CoV Mix	50 µl	125 µl
5x One-step Probe CoV Mix	400 µl	1.0 ml
20x SARS-CoV-2 Primer/Probe Mix (N/E/RdRP/RP)	100 µl	250 µl
SARS-CoV-2 Positive Control (N/E/RdRP)	80 µl	2 x 80 µl
Water, nuclease free	1.25 ml	5.0 ml

Mix components:

Mix name	Description
40x One-step SOLIScript® CoV Mix	SOLIScript® Reverse Transcriptase, RiboGrip™ RNase Inhibitor.
5x One-step Probe CoV Mix	HOT FIREPol® DNA polymerase, reaction buffer, dNTPs, 15 mM $MgCl_2$ (1x RT-qPCR solution – 3 mM).
20x SARS-CoV-2 Primer/Probe Mix (N/E/RdRP/RP)	Primers and fluorescently labelled probes for SARS-CoV-2 regions in N (FAM), E (HEX), RdRP (ROX) genes, human RNase P mRNA (Cy5).
SARS-CoV-2 Positive Control (N/E/RdRP)	dsDNA Positive Control containing targets specific to the SARS-CoV-2 genomic regions targeted by the assays.

Step-by-step guidelines:

1. All reagents and samples should be thawed completely at room temperature, mixed (by inverting, pipetting or gentle vortexing) and centrifuged briefly before use.
2. Determine the total number of reactions per assay run. In addition to test RNA samples, each assay should include:
   - one Positive Control (PC) using the SARS-CoV-2 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.
3. Combine the components for the number of reactions required plus 10% overage to compensate for pipetting errors. Prepare a reaction master mix at room temperature. For multiple reactions, recommended pipetting order is as follows: nuclease-free water, 20x SARS-CoV-2 Primer/Probe Mix, 5x One-step Probe CoV Mix, 40x One-step SOLIScript® CoV Mix.

Reaction mix component	Volume per reaction
5x One-step Probe CoV Mix	4.0 µl
40x One-step SOLIScript® CoV Mix	0.5 µl
20x SARS-CoV-2 Primer/Probe Mix (N/E/RdRP/RP)	1.0 µl
Water, nuclease free	6.5 µl

4. Mix the reaction mix thoroughly, centrifuge briefly and distribute 12 µl of the reaction master mix to each reaction well according to the plate setup.

5. Add 8 µl of RNA sample, Negative Control and Positive Control to the appropriate wells according to the plate setup.

6. Seal the plate with an appropriate seal, centrifuge reactions briefly, and place in the real-time PCR instrument.

7. Set up and run the real-time PCR instrument. Set the thermal protocol as follows.

Step	Temperature	Time	
Reverse transcription	50°C	10 min	
Initial activation	95°C	10 min	
Denaturation	95°C	3 sec	40 cycles
Annealing/Elongation[1]	62°C	15 sec	

[1] Acquisition must be performed at the end of this stage.

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

- FAM (N gene)
- VIC/HEX/JOE (E gene)
- Cal Red 610/ROX/Texas Red (RdRP gene)
- Quasar 670/Cy5 (RNase P)

Compatible real-time instruments

- The Kit is compatible with ROX-independent and ROX-dependent qPCR platforms.

- NOTE: If using ROX-dependent qPCR cycler, ROX channel should be deactivated and set to "none" while selecting passive reference that will be used in the assay.

Analysis of results

Before interpreting test sample results, it is necessary to verify the success of the run. Testing needs to be repeated if the following criteria are not satisfied:

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

The specific software for the real-time PCR cycler employed must be used to analyse amplification results. It is recommended to use the Auto Baseline and the Auto Threshold in the analysis settings.

Please manually inspect amplification curves for all samples assigned a Cq value to verify the positive amplification.

If all the control acceptance criteria are fulfilled, then each sample can be assessed with the interpretation matrix on the Page 7.

Interpretation of results

Target				
N, FAM	E, HEX	RdRP, ROX	RNase P, Cy5	Decision
POS	POS	POS	POS/NEG	SARS-CoV-2 detected
POS	POS	NEG	POS/NEG	SARS-CoV-2 detected
POS	NEG	POS	POS/NEG	SARS-CoV-2 detected
NEG	POS	POS	POS/NEG	SARS-CoV-2 detected
NEG	NEG	NEG	POS	SARS-CoV-2 not detected
NEG	NEG	NEG	NEG	Sample retesting*
POS	NEG	NEG	POS/NEG	Sample retesting*
NEG	POS	NEG	POS/NEG	Sample retesting*
NEG	NEG	POS	POS/NEG	Sample retesting*

* Sample retesting needs to be performed by extraction of RNA from the specimen and repeating RT-qPCR. If the retesting result remains inconclusive, collection and testing of a new sample should be considered.

Safety warnings and precautions

This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing, such as laboratory overalls, gloves, and safety glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water. Refer to Safety Data Sheet for more information.

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.

Technical support

Contact your sales representative for any questions or send an email to support@solisbiodyne.com

DS-08-83 v1
Effective date: 02.02.2022

Manufactured by: Solis BioDyne OÜ, Teaduspargi 9, 50411 Tartu, Estonia (EU). www.solisbiodyne.com