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

Catalogue Number	Size (20 µl reactions)
08-82-00100	100 reactions
08-82-00250	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 1 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 assay optimized for RT-qPCR detection of SARS-CoV-2 viral RNA extracted from clinical samples or extracted from any other sample containing SARS-CoV-2 viral RNA. 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.
- 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, VIC/HEX/JOE channel) and RNA-dependent RNA polymerase gene (RdRP, Cal Red 610/ROX/Texas Red channel), as well as human RNase P transcript (RPP30, Quasar 670/Cy5 channel).
- Human RNase P primers and probe set is designed to detect exclusively mRNA transcripts (genomic DNA is not amplified) and serves as an internal control to monitor sample quality and RT-qPCR amplification.
- 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
Water, Nuclease-free	1.25 ml	5.0 ml

Mix components:

Mix component	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 designed for SARS-CoV-2 regions in N (FAM channel), E (VIC/HEX/JOE channel), RdRP (Cal Red 610/ROX/Texas Red channel) genes, as well as human RNase P mRNA transcripts (Quasar 670/Cy5 channel).

Step-by-step guidelines:

1. Thaw One-Step Probe CoV Mix, One-step SOLIScript® CoV Mix, SARS-CoV-2 Primer/Probe Mix, template RNA, and nuclease-free water. Mix each component by gentle vortexing or pipetting up and down, then centrifuge briefly.
2. Prepare a reaction mix. Add all required components except the template RNA.

Component	Volume[1]	Final conc.
5x One-step Probe CoV Mix	4.0 µl	1x
40x One-step SOLIScript® CoV Mix	0.5 µl	1x
20x SARS-CoV-2 Primer/Probe Mix (N/E/RdRP/RP)	1.0 µl	1x
RNA sample	5–10 µl	
Water, Nuclease-free	up to 20 µl	
Total reaction volume	20 µl	

[1] Scale all components proportionally according to sample number and reaction volumes. Make sure to use enough of each reagent for your reactions, plus 10% extra volume to accommodate pipetting errors. For high reaction efficiency, do not exceed 20 µl reaction volumes. When preparing a reaction master mix for multiple reactions, recommended pipetting order is as follows: nuclease-free Water, 20x SARS-CoV-2 Primer/Probe Mix (N/E/RdRP/RP, 5x One-step Probe CoV Mix, 40x One-step SOLIScript® CoV Mix.

3. Mix the reaction mix thoroughly, then centrifuge briefly. Dispense appropriate volumes of mix into PCR wells.

4. A negative (no template) PCR control is needed to eliminate the possibility of sample contamination on the assay run and must be used on every assay plate. This control is molecular grade, nuclease-free water.

5. Add template RNA to the PCR wells. Seal the wells using the procedure recommended for the cycling instrument being used and centrifuge the reactions briefly.

6. Incubate your PCR reactions in thermal cycler as follows:

Cycle step	Temperature	Time	
Reverse transcription	50°C	10 min	
Initial activation[1]	95°C	10 min	
Denaturation	95°C	3 sec	40 cycles
Annealing/Elongation[2]	62°C	15 sec	

[1] 10 min initial incubation is crucial for the full activation of the HOT FIREPol® DNA Polymerase.

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

Plate settings

- 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

1. Make sure that there is no amplification in negative PCR controls in any of the fluorescence channels (FAM, VIC, Texas Red, Cy5 channels). If amplification is detected in a negative control, it is recommended to repeat the assay to rule out the accidental contamination.

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

See interpretation of results on 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 clinical sample 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.

Technical support

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

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