

SOLIScript® 1-step CoV Kit

Supplementary Data Sheet

Specifically adjusted protocols for Charité (Germany)^{1,2}
and CDC (USA)³ diagnostic assays

Revised 06.04.2020

General notes:

1. The Kit has been tested and validated by diagnostic laboratories using primer-probe panels recommended by Charité, (Germany)^{1,2} and CDC (USA)³ protocols according to WHO "Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans", section: "Molecular assays to diagnose COVID-19"; subsection: "In-house developed molecular assays".
2. SARS-CoV-2 primers and probes, applicable positive and internal extraction controls are not provided with the Kit and should be supplied by the user. Being listed in this document does not imply any endorsement or scientific and diagnostic validation by Solis BioDyne.
3. To avoid repeated freezing and thawing as well as to minimize the contamination risk of stock solutions of reagents, it is highly recommended to divide large-volume stocks into several smaller aliquots and store them at -20 °C.
4. For multiple reactions, preparation of a master mix of common components is crucial to reduce pipetting errors. Scale all components proportionally according to sample number and reaction volumes. Make sure you use enough of each reagent for your reactions, plus 10% extra volume to accommodate pipetting errors.

Thermal conditions recommended for Charité (Germany) and CDC (USA) diagnostic assays

Thermal conditions compatible with most cyclers:

Step	Temp.	Time	
Reverse transcription	55 °C	10 min	
Initial denaturation	95 °C	10 min	
Denaturation	95 °C	15 sec	45 cycles
Annealing/Elongation	58 °C	30 sec	

Alternative thermal conditions:

Step	Temp.	Time	
Reverse transcription	48 °C	30 min	
Initial denaturation	95 °C	10 min	
Denaturation	95 °C	5 sec	45 cycles
Annealing/Elongation	55 °C	30 sec	

¹ Corman et al. 2020 „Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR“, doi: 10.2807/1560-7917.ES.2020.25.3.2000045

² Adapted from https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf?sfvrsn=de3a76aa_2

³ CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (<https://www.fda.gov/media/134922/download>)

1. Diagnostic detection of SARS-CoV-2 (2019-nCoV) using Charité, Berlin Germany protocol

Assay/Use	Oligonucleotide ID	Sequence (5'–3')	Comment
RdRP gene	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRP_SARSr-R1	CARATGTAAASACACTATTAGCATA	Use 800 nM per reaction
	RdRP_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe, will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
E gene	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nM per reaction
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	Use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nM per reaction

1.1 Reaction Mix for First line screening assay (E assay)

Component	Volume	
Nuclease-free water	8.5 µl	
5x One-step Probe CoV Mix	4 µl	
40x One-step SOLIScript® CoV Mix	0.5 µl	
Primer E_Sarbeco_F1 (10 µM stock solution)	0.8 µl	ACAGGTACGTTAATAGTTAATAGCGT
Primer E_Sarbeco_R2 (10 µM stock solution)	0.8 µl	ATATTGCAGCAGTACGCACACA
Probe E_Sarbeco_P1 (10 µM stock solution)	0.4 µl	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
Template RNA, add	5 µl	
Total reaction volume	20 µl	

1.2 Reaction Mix for Confirmatory assay (RdRP assay; detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs)

Component	Volume	
Nuclease-free water	7.3 µl	
5x One-step Probe CoV Mix	4 µl	
40x One-step SOLIScript® CoV Mix	0.5 µl	
RdRP_SARSr-F2 (10 µM stock solution)	1.2 µl	GTGARATGGTCATGTGTGGCGG
RdRP_SARSr-R1 (10 µM stock solution)	1.6 µl	CARATGTTAAASACACTATTAGCATA
RdRP_SARSr-P1 (10 µM stock solution)	0.2 µl	FAM- CCAGGTGGWACRTCATCMGGTGATGC-BBQ
RdRP_SARSr-P2 (10 µM stock solution)	0.2 µl	FAM-CAGGTGGAACCTCATCAGGAGATGC- BBQ
Template RNA, add	5 µl	
Total reaction volume	20 µl	

1.3 Reaction Mix for Discrimatory assay (RdRp assay, specific for 2019-nCoV)

Component	Volume	
Nuclease-free water	7.5 µl	
5x One-step Probe CoV Mix	4 µl	
40x One-step SOLIScript® CoV Mix	0.5 µl	
RdRP_SARSr-F2 (10 µM stock solution)	1.2 µl	GTGARATGGTCATGTGTGGCGG
RdRP_SARSr-R1 (10 µM stock solution)	1.6 µl	CARATGTTAAASACACTATTAGCATA
RdRP_SARSr-P2 (10 µM stock solution)	0.2 µl	FAM-CAGGTGGAACCTCATCAGGAGATGC- BBQ
Template RNA, add	5 µl	
Total reaction volume	20 µl	

2. Diagnostic detection of SARS-CoV-2 (2019-nCoV) using CDC (US) protocol

Name	Description	Sequence (5'-3')	Working conc.
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GACCCCAAATCAGCGAAAT-3'	20 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCTGGTACTGCCAGTTGAATCTG-3'	20 µM
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3'	5 µM
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTACAAACATTGGCCGCAA-3'	20 µM
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCGCGACATTCCGAAGAA-3'	20 µM
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACAATTTGCCCCAGCGCTTCAG-BHQ1-3'	5 µM
RP-F	RNAse P Forward Primer	5'-AGATTTGGACCTGCGAGCG-3'	20 µM
RP-R	RNAse P Reverse Primer	5'-GAGCGGCTGTCTCCACAAGT-3'	20 µM
RP-P	RNAse P Probe	5'-FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ1-3'	5 µM

Reaction Mix for 2019-nCoV_N1, 2019-nCoV_N2 and RP singleplex assays

Component	Volume	Final concentration
Nuclease-free water	9 µl	
5x One-step Probe CoV Mix	4 µl	1x
40x One-step SOLIScript® CoV Mix	0.5 µl	1x
Primer F (20 µM stock solution) ¹	0.5 µl	500 nM
Primer R (20 µM stock solution) ¹	0.5 µl	500 nM
Probe (5 µM stock solution) ¹	0.5 µl	125 nM
Template RNA, add	5 µl	
Total reaction volume	20 µl	

¹ If a Combined Primer/Probe Mix is used, pipet 1.5 µl of Combined Primer/Probe Mix per reaction.