Synbio Technologies
Genes for Life

SARS-CoV-2 Real-time RT-PCR Detection Assay

For Research Use Only

Catalog# SYN202000
(This Assay Kit Includes Box #1 and Box #2)

25 Tests, 100 Reactions

Materials Provided

Box #1: Primers and Probes

Reagent Label	*Part #*	*Description*	*Quantity / Tube*	*Reactions / Tube*
SARS-CoV-2_N1	ST202001	SARS-CoV-2_N1 Combined Primer/Probe Mix	37.5 µL/0.56 nmol	25
SARS-CoV-2_N2	ST202002	SARS-CoV-2_N2 Combined Primer/Probe Mix	37.5 µL/0.56 nmol	25
SARS-CoV-2_N3	ST202003	SARS-CoV-2_N3 Combined Primer/Probe Mix	37.5 µL/0.56 nmol	25
RP	ST202004	Human RNase P Forward Primer/Probe Mix	37.5 µL/0.56 nmol	25
2X Reaction Buffer	ST202006	Buffer, dNTP	1.2 ml	100
Enzymes Mix	ST202007	UDG, Taq DNA Polymerase, Reverse Transcriptase, RNase inhibitor	200 µL	100
Nuclease-free Water	ST202008	Nuclease-free Water	1.5 ml	100

Box #2: Positive Control

Reagent Label	*Part #*	*Description*	*Quantity*	*Notes*
SARS-CoV-2 Positive Control (Plasmid DNA)	ST202005	The assays target regions within the SARS-CoV-2 nucleocapsid gene which is present in the SARS-CoV-2_N Positive Control plasmid. The Hs_RPP30 Control contains a portion of the RPP30 gene, a gene present in the human genome.	125 µL/1 tube	Provides (25) 5 µL test reactions

Reagent Storage, Handling, and Stability

- Store all primers and probes and the positive control at -20°C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.

- Primers, probes, buffer, enzyme mix and positive control must be thawed and kept on a cold block at all times during use.

Methods

1. Reaction mix (in reagent preparation area)
   1.1 Thaw 2X Reaction Buffer Mix prior to use.
   1.2 Mix buffer, enzyme mix, and primer/probes by inversion 5 times.
   1.3 Centrifuge buffer and primers/probes for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
   1.4 Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the NTC, Positive Control RP reactions and for pipetting error. Use the following guide to determine N:
   1.5 If number of samples (n) including controls equals 1 through 14, then N = n + 1
   1.6 If number of samples (n) including controls is 15 or greater, then N = n + 2
   1.7 For each primer/probe set, calculate the amount of each reagent to be added for each reaction mixture (N = # of reactions).

1-Step RT-qPCR Master Mix

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Nuclease-free Water	N x 1.5 µL
2	Combined Primer/Probe Mix	N x 1.5 µL
3	2X Reaction Buffer Mix	N x 10.0 µL
4	Enzymes Mix	N x 2.0 µL
	Total Volume	N x 15.0 µL

- Dispense reagents into each respective labeled 1.5 mL microcentrifuge tube. After addition of the reagents, mix reaction mixtures by pipetting up and down. Do not vortex.
- Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
- Set up reaction strip tubes or plates in a 96-well cooler rack.
- Dispense 15 µL of each master mix into the appropriate wells.

2. Sample preparation (In sample preparation area)

2.1 Nucleic acid extraction

The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the panel:

Qiagen QIAamp® DSP Viral RNA Mini Kit
Recommendation(s): Utilize 100 µL of sample and elute with 100 µL of buffer or utilize 140 µL of sample and elute with 140 µL of buffer.

2.2 Sample loading

Add 5ul of the testing RNA sample, any of the positive control or negative control respectively, to reach a total volume of 20µl/tube. Seal the cap of the tube and centrifuge at 1000rpm for 15 seconds.

3. PCR and measurement (PCR area)

Place the PCR tube into real-Time PCR System, and follow the protocol below:

Step	Temperature	Time	Cycle #
UDG digestion	25℃	2min	1
Reverse transcript	50℃	15min	1
Pre-denature	95℃	2min	1
Denature	95℃	10s	45
Amplification	55℃	30s	

Data Analysis

Data can be interpreted as follows if the quality control standard mentioned above is met.

1. Positive: Sample Ct in both fluorescent channels should be positive before or at 37 cycles, with an S-shaped curve including log-phase.
2. Negative: All fluorescent channels have Ct > 40, or no signal, the result is considered negative.
3. When to repeat the assay: If one of the fluorescent channels has Ct value ≤37, while the other channel Ct value between 37-40, we suggest repeating the test to confirm. If the repeated test still shows 37<Ct≤40, and the S-shaped curve shows the log phase, the result can be considered as positive.

SARS-CoV-2-N1 <40cycle	**SARS-CoV-2-N2 <40cycle**	**SARS-CoV-2-N3 <40cycle**	**RP <40cycle**	**Result Interpretation**
+	+	+	±	SARS-CoV-2 Detected
If only one, or two, of the three targets is positive			±	Inconclusive Result
-	-	-	+	SARS-CoV-2 Not detected
-	-	-	-	Invalid Result

Limitations and Precautions

- The assay is available for research use only (RUO).
- Performance of the assay is impacted by a range of uncontrolled and un-tested factors such as sample quality, sample cross-contamination, and variations in sample extraction methods and data analysis.
- The assay's design is based on the public SARS-Cov-2 genome sequence, if the virus mutates in the RT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the sample 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.
- Maintain separate areas for assay setup and handling of nucleic acids.
- 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.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- 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.
- 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.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Primers, probes, and enzyme master mix must always be thawed and maintained on cold block during preparation and use.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- The samples used in the test are considered as infectious biohazardous material; handle all samples using safe laboratory procedures. Refer to Biosafety in Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition by CDC or any applicable biosafety regulation. Dispose of unused kit reagents and samples according to local, state, and federal regulations.