

Protocol of IQ REAL™ SARS-CoV-2 Qualitative System

A. Reagent contents: (192 tests/package):

1. Premix Pack (containing dNTPs, primers, probe, and IQzyme DNA polymerase)	3 x 8 tube/pack (8 rxn/tube)
2. Premix Buffer B	4 x 1.3 ml/vial
3. P(+) Control	1 x 1 pellet/vial
4. P(+) Control Buffer	1 x 110 µl/vial

B. Nucleic acid extraction:

- 1) taco™ mini Automatic Nucleic Acid Extraction System is recommended for nucleic acid extraction.
- 2) Please refer to the user manual of taco™ mini Automatic Nucleic Acid Extraction System and taco™ Preloaded DNA/RNA Extraction Set. Ensure that correct volumes of supernatant/sample input and Eluting Buffer are used, please refer to the table below.

Sample type	Volume	
	Supernatant/ Sample input	Eluting Buffer
Swab	200 µl	200 µl

C. Operation procedure:

- 1) Before Reaction (for the first time use):
 - a) Add 207 µl Premix Buffer B to reconstitute Premix pellet. The volume, compensated for pipetting error, is sufficient for 9 reactions.
 - If the thermal cycler is capable of detecting for ROX™ Reference Dye, please refer to its user manual and adjust ROX™ concentration. Add 4.5 µl 25 µM ROX™ Reference Dye into Premix tube (High ROX) and add 4.5 µl 2.5 µM ROX™ into Premix tube (Low ROX).

ROX™	ROX™ Stock concentration	ROX™ final concentration	Dilution factor
High	25 µM	500 nM	50X
Low	2.5 µM	50 nM	50X

- b) Add 100 µl P(+) Control Buffer to P(+) Control and store the reconstituted P(+) Control in 2-8°C.

2) Reagent Preparation (for one reaction):

Premix	23 μ l
Nucleic acid sample or P(+) Control	2 μ l
Total volume	25 μ l

D. Real-time PCR program:

42° C, 30 minutes; followed by 95° C, 15 seconds and 60° C, 1 minute, repeat 40 cycles.

Target	Reporter	Quencher
SARS-CoV-2	FAM	BHQ1
Internal Control	VIC	BHQ1

E. Result interpretation:

- For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.
- Positive Control: The results are valid when Ct value of positive control is between 22-28.

Detection Channel		Data interpretation
FAM	VIC	
+	+	SARS-CoV-2 (target <i>orf 1ab</i> gene) RNA detected.
+	—	SARS-CoV-2 (target <i>orf 1ab</i> gene) RNA detected.
—	+	SARS-CoV-2 (target <i>orf 1ab</i> gene) RNA not detected.
—	—	PCR inhibition or reagent failure. Retest the sample.

F. Analytical sensitivity and specificity:

The detection limit is about 10 copies/reaction. This reagent can detect SARS-CoV-2, and does not detect other pathogens, such as bat SARS-like-CoV, human coronavirus HKU1, MERS-CoV, influenza virus A, influenza virus B.

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For any questions, please contact GeneReach Technical Service Department:

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