BlazeTaq™ Probe SARS-CoV-2 One-Step RT-qPCR Detection Kit (for S and N gene detection)

Easy-to-use mixes for probe-based real-time RT-qPCR

With ROX Reference Dye	Without ROX Reference Dye
Cat.No.**QP201** (20 µl × 200 reactions)	Cat.No.**QP203** (20 µl × 200 reactions)
Cat.No.**QP202** (20 µl × 1000 reactions)	Cat.No.**QP204** (20 µl × 1000 reactions)

Performance optimized for All-In-One™ qPCR Primers

User Manual

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USER MANUAL

BlazeTaq™ Probe SARS-CoV-2 One-Step RT-qPCR Detection Kit (for S and N gene detection)

I. Description

The BlazeTaq™ Probe SARS-CoV-2 One-Step RT-qPCR Detection Kit uses reverse transcription PCR (RT-qPCR) for SARS-CoV-2 related S and N gene detection, using RNA as starting material. It offers a convenient master mix to convert RNA to DNA, then amplify, all in a one-step reaction. The kit is supplied with 5X Probe One Step RT-qPCR Buffer with dNTP and all required buffer components and a 50X RTU Mix with reverse transcriptase and Hot-Start Taq DNA polymerase, and is universally compatible with all instrument platforms. This kit uses a reverse transcriptase to convert RNA to DNA, and an antibody-modified Taq DNA polymerase, avoiding polymerase activity prior to thermal cycling. Upon heating to 95 ºC for 30 s, the antibody dissociates, and full activity of the Taq DNA polymerase is restored. The optimized buffer system allows high amplification efficiency and specificity, as well as enhanced sensitivity of real time PCR reactions over a wide range of template concentrations.

Features:

1. Contains UDG/dUTP to reduce carry-over contamination.
2. High sensitivity. Can detect as few as 10 copies of Sars-CoV-2 genomic RNA.
3. Broad-spectrum detection. Proprietary primer/probe designs enable detection of most SARS-CoV-2 strain variants.

II. Related Products

GeneCopoeia offers comprehensive solutions for studying gene expression. Precise co- development ensures that these products work well together and provide robust and reproducible results.

Product	Description
BlazeTaq™ SYBR® Green qPCR mix 2.0	SYBR Green-based real-time quantitative PCR Mix
SureScript™ First-Strand cDNA Synthesis Kit	Reverse transcribe mRNA into first–stand cDNA
All-in-One™ qPCR Primers	Validated, gene-specific primers ensure specificity and sensitivity (human, mouse and rat)
ExProfile™ Gene qPCR Arrays	High-throughput or focused group profiling of gene expression
All-in-One™ miRNA First-Strand cDNA Synthesis Kit	Reverse transcribe miRNA into first –stand cDNA
All-in-One™ miRNA qRT-PCR Detection Kits	SYBR Green-based detection kit accurately quantifies miRNA expression

All-in-One™ miRNA qPCR Primers	Validated human, mouse, rat miRNA primers for robust, reproducible and reliable quantitation of miRNA activity
miProfile™ miRNA qPCR Arrays	High-throughput or focused group profiling of miRNA expression
RNAzol® RT RNA Isolation Reagent	Easy isolation of mRNA, microRNA or total RNA

III. Contents and Storage

Contents and storage recommendations for the BlazeTaq™ Probe SARS-CoV-2 One-Step RT-qPCR Detection Kit (for S and N gene detection) are provided in the following table.

For kits with the catalog number **QP201** and **QP202**

Catalog Number	Contents	Quantity	Storage temperature/ conditions
QP201-01	BlazeTaq™ Probe One Step RT-qPCR Buffer (with dUTP) (5×)	1×800 μL 5×(1×800 μL)	Store at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP201-02	BlazeTaq™ RTU Mix (50×)	1×80 μL 5×(1×80 μL)	Store at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP201-03	SARS-CoV-2 Primer/Probe Mix SN (20×)	1×200 μL 5×(1×200 μL)	Store in dark at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP201-04	SARS-CoV-2 Positive Control	1×250 μL 5×(1×250 μL)	Store at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP001-02	ROX Reference Dye (30 μM)	1×80 μL 5×(1×80 μL)	Store in dark at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP201-05	ddH_2O	2×1.8 mL 5×(2×1.8 mL)	Store at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.

Contents and storage recommendations for the BlazeTaq™ Probe SARS-CoV-2 One-Step RT-qPCR Detection Kit (for S and N gene detection) (without ROX) are provided in the following table.

For kits with the catalog number **QP203** and **QP204**

Catalog Number	Contents	Quantity	Storage temperature/ conditions
QP201-01	BlazeTaq™ Probe One Step RT-qPCR Buffer (with dUTP) (5×)	1×800 μL 5×(1×800 μL)	Store at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP201-02	BlazeTaq™ RTU Mix (50×)	1×80 μL 5×(1×80 μL)	Store at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP201-03	SARS-CoV-2 Primer/Probe Mix SN (20×)	1×200 μL 5×(1×200 μL)	Store in dark at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated

			freezing/thawing.
QP201-04	SARS-CoV-2 Positive Control	1×250 μL 5×(1×250 μL)	Store at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP201-05	ddH_2O	2×1.8 mL 5×(2×1.8 mL)	Store at -20°C (stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.

Required Materials (Not Included)

- RNA template
- PCR strip tubes or microcentrifuge tubes (for reaction setup)
- Nuclease-free pipettes
- Microcentrifuge
- qPCR instrument

IV. Preparation

Wearing a lab coat, disposable gloves and protective goggles are recommended when handling chemicals.

RNA Sample Preparation

Follow the manufacturer's manuals. The following RNA extraction reagents are recommended:

1. Genecopoeia: RNAzol® RT RNA Isolation Reagent (cat. # QP020)
2. Qiagen: QIAamp Viral RNA Mini Kit.
3. Roche: High Pure Viral RNA Kit
4. Takara: NucleoSpin® RNA Virus kit.

IMPORTANT NOTES:

1. Store the kit at －20° C. Avoid storage or leaving reagents at 4° C or room temperature. Avoid light exposure at all times.
2. Mix reagents thoroughly by gently inverting tubes several times while avoiding bubbles, and then briefly centrifuge before use.
3. Prepare the reaction mix with PCR grade water.
4. RT-qPCR is a sensitive RNA detection method. Set up all reactions on ice to reduce risk of RNA degradation.
5. A denaturation or melt curve step should be added at the end of the RT-qPCR cycling protocol to evaluate amplification specificity.
6. Read all procedures before setting up the PCR reaction.

V. Procedure

1. Thaw the BlazeTaq™ components at room temperature, then place on ice. After thawing completely, briefly mix each component by inversion, pipetting or gentle vortexing.

2. Prepare the RT-qPCR reaction **Master Mix** according to the table below in a RNase-Free tube. Mix well, briefly spin and put on ice.

Reagent	Each Rxn [a]	Multiple Rxn [b]
BlazeTaq™ Probe One Step RT-qPCR Buffer (with dUTP) (5×)	4 μL	4 μL x n
BlazeTaq™ RTU Mix (50×)	0.4 μL	0.4 μL x n
SARS-CoV-2 Primer/Probe Mix SN (20×)	1 μL	1 μL x n
ROX Reference Dye[c] (30 μM), optional	0.4 - 0.1 μL	0.4 - 0.1 μL x n
Final (add ddH2O to)	8 μL	8 μL x n

3. Prepare samples and controls in each of PCR reaction tubes or plates:

- **SARS-Cov-2 samples:** Purified RNA from SARS-Cov-2 samples and dissolved in ddH_2O.
- **SARS-Cov-2 Positive Controls (PC)**: Included in kit. For RT-PCR validation.
- **No-Template Controls (NTC)**: To verify contamination of the reaction mixes, just add ddH_2O.

Reagent	Volume	Add ddH_2O to final
SARS-CoV-2 RNA samples [d]	5-12 μL	12 μL
SARS-Cov-2 Positive Controls (PC)	5 μL	12 μL
No-Template Controls (NTC)	NA	12 μL

Note:

a. The kit has been optimized for a final reaction volume of 20 μL. If the total reaction volume is changed, maintain each component in the proper proportion.

b. Prepare a little more Master Mix than needed to avoid shortage due to pipetting errors.

c. ROX Reference Dye is only supplied in the BlazeTaq™ Probe SARS-CoV-2 One-Step RT-qPCR Detection Kit (for S and N gene detection) (Cat. No. QP201 and QP202). It should be added only for qPCR instruments that require ROX for calibration.

ROX Reference Dye provides an internal reference to which the reporter-dye signal can be normalized during data analysis. Normalization is necessary to correct for fluorescence fluctuations due to changes in concentration or volume. Adjust the ROX Reference Dye to optimal concentration according for each relevant qPCR instrument.

Instrument	ROX per 20 µl PCR Reaction	Final Concentration
BioRad iCycler, MyiQ, iQ5, CFX-96, CFX-384, Eppendorf Mastercycler realplex, Roche LightCycler 480, LightCycler 2.0	None	No ROX
ABI PRISM 7000/7300/7700/7900HT and 7900HTFast, ABI Step One, ABI Step One Plus	0.4 µl (0.2-0.4 µl)	600 nM (300-600 nM)
ABI 7500, 7500 Fast, ABI ViiA7, Stratagene Mx3000P, Mx3005P, Mx4000	0.1 µl (0.02-0.1 µl)	150 nM (30-150 nM)

For other instruments that need calibration with ROX but have not been listed in the table, please optimize the concentration of ROX according to the guidelines of the specific instrument.

d. RNA samples should be dissolved in ddH_2O or 10mM Tris-Cl pH7.4. The added RNA can be from 0.01 ng to 100 ng, depending on target abundance and RNA quality. Low purity of RNA samples could affect the amplification and cause false negative results.

4. Add 8ul of the RT-qPCR reaction Master mix (from Step 2) to each of the PCR reaction tubes or plates with RNA samples or controls. Cover the tubes and briefly mix.

5. Briefly centrifuge to remove bubbles and make sure all the reagents are at the bottom of the reaction tubes/plates.

6. The following program of the RT-qPCR reaction is recommended:

Cycles	Steps	Temperature	Time	Detection	Channels
1	UDG digestion	25°C	5min	No	
1	Reverse Transcription	52°C	10 min	No	
1	Initial Denaturation	95°C	60 sec	No	
45	Denaturation	95°C	10 sec	No	
	Extension	60°C	30 sec	Yes	Fam, Hex, Cy5

Notes

- In the RT-qPCR reaction, use the channels **Fam (S gene), Hex (N gene) and Cy5 (GAPDH internal control).**
- 60° C is the recommended default extension temperature in the reaction system, but can be adjusted in the range of 58 - 65 ° C if using other primer-probe sets.
- The above reaction is based on the iQ5 qPCR instrument manual from Bio-Rad. If a different qPCR instrument is used, please reference the relevant instrument manual and adjust the extension time and melting curve conditions accordingly.

VI. Result Analysis

Validation of RT-PCR: The Ct of the SARS-Cov-2 Positive Controls (PC) should be < 30 (Fam, Hex and Cy5). The Ct of the No-Template Control (NTC) should not be detectable or ≥ 40. Otherwise, the RT-PCR is not valid.

Ct value	Fam (S)	Hex (N)	Cy5 (GAPDH)	Result
PC	< 30	< 30	< 30	RT-PCR is validated.
NTC	≥ 40	≥ 40	≥ 36	
Samples	< 40	< 40	< 36	Positive
Samples	≥ 40	≥ 40	< 36	Negative
Samples	≥ 40	< 40	< 36	Suspicious, need to repeat RT-PCR and/or re-collect samples
Samples	< 40	≥ 40	< 36	
Samples	≥ 40	≥ 40	≥ 36	Sample RNA is too low. Re-collect samples and repeat RT-PCR.
Samples	≥ 40	< 40	≥ 36	
Samples	< 40	≥ 40	≥ 36	

VII. Troubleshooting Guide

Problem	Possible Cause	Solution
No or low RT-qPCR product (signal)	RNA template has been damaged/degraded.	Follow the RNA isolation kit procedure carefully, always wearing a lab coat, gloves and mask when working with RNA and use RNA-Grade reagents and materials. Check the RNA quality by RNA electrophoresis in a denaturing gel.
	An inhibitor was present in the RNA template.	Trace amounts of an inhibitor, such as guanidine salts, in the RNA template can inhibit the cDNA synthesis. Re-precipitate the RNA with ethanol and wash the pellet with 75% ethanol.
	There is not enough RNA template.	After increasing the number of cycles has shown no success, repeat the reaction with a higher concentration of template.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
Product detected at lower-than expected cycle number, and/or positive signal from no template controls	RNA template or RT-qPCR reaction carryover contamination.	Although the kit contains reagents to reduce carry-over contamination, precautions are still needed to be taken, such as maintaining aseptic conditions. Use separate dedicated pipettes for RT-qPCR reactions. Assemble reactions (except for target addition) in a DNA-free area.

VIII. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to use of all BlazeTaq™ Probe SARS-CoV-2 One-Step RT-qPCR Detection Kit (for S and N gene detection) (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any
GeneCopoeia products, please contact us at 301-762-0888.

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