Biomiga Inc.
The Inventor of EZgene™ and ViraTrap™ Systems

COVID-19 One-Step RT-qPCR System, No ROX Catalog No. QP1318-03 **Store at -20°C**

Biomiga One-Step qRT-PCR System, No ROX is stable for 1 year when stored at -20°C. No significant performance loss was detected after 10 times freeze-thaw cycles. For convenience, it may be stored at 4°C for up to two weeks.

Description
COVID-19 One-Step qRT-PCR System, No ROX or Low Rox is consist of Enzyme Mix and 2X Reaction Mix, It is designed for the reverse transcription (RT) and polymerase chain reaction (PCR) amplification of a specific target RNA from either total RNA or mRNA in a single tube for the detection and quantification of RNA using real-time detection instruments. The proprietary Enzyme Mix contains an engineered Script Reverse Transcriptase (RNaseH minus), Hot Start *Taq* DNA polymerase, stabilizers. 2X Reaction Mix consists of a proprietary buffer system, 6 mM $MgCl_2$, dNTPs, enhancers and stabilizers. It also contains an optimal concentration of ROX for instruments that require low ROX for a reference signal. Please consult our website and Instrument Compatibility section below for the correct product for your real-time PCR system.

Hot Start *Taq* DNA polymerase is an antibody-inactivated hot-start enzyme designed to block polymerase activity between ambient to RT reaction temperature. RnaUsScript RT enzyme can synthesize cDNA at a temperature range of 40-55°C. Once the PCR step reaches denaturation temperature (95°C), *Taq* DNA polymerase activity is restored and the resulting PCR exhibits higher sensitivity, specificity and yield.

The system enables highly sensitive detection from as few as 10 copies of SARS-CoV-2 target genes (N1 and N2), with a broad dynamic range that supports accurate quantification of mRNA copies. Sufficient reagents are provided for 125, 500, or 1,250 amplification reactions of 20 µl each.

Features
- Easy-to-use two tube system: Enzyme Mix (50X) and Reaction Mix (2X)
- Broad range of RT reaction temperature (40-55°C)
- Broad dynamic range of detection
- Hot start PCR system
- Fully optimized RT-qPCR buffer and robustness
- High sensitivity, specificity, and reproducibility
- Ideal for probe based COVID-19 viral RNA detection and quantitation

Product qualification
Biomiga One-Step qRT-PCR System demonstrates high RT-qPCR efficiency and linear resolution over a wide linear dynamic range. Stringent specifications are maintained to ensure lot to lot consistency. This product is free of detectable DNase and RNase activities.

Kit components

	1,250 Rxns
• **One-Step qRT-PCR Reaction Mix (2X)**	1.25 ml X10
• **One-Step qRT-PCR Enzyme Mix (50X)**	500 µl

Recommended qRT-PCR reaction assembly

The following protocol is suggested as a starting point. For multiple reactions, prepare a master mix of common components by adding the appropriate volume to each tube or plate well on ice, and then add the unique reaction components (*e.g.,* RNA template).

1. Thaw all components at room temperature. Hand warming can facilitate the process. Mix gently, then centrifuge to collect contents to the bottom of the tube before using. Place all components on ice after thawing.

2. Assemble the reaction on ice.

Components	20 µl Rxn	Final Concentration
One-Step RT-qPCR Reaction Mix, No ROX (2X)	10 µl	1X
One-Step RT-qPCR Enzyme Mix Mix (50X)	0.4 µl	1X
Forward Primer (10 µM)	0.4 µl	200nM (Variable 100-900 nM)
Reverse Primer (10 µM)	0.4 µl	200nM (Variable 100-900 nM)
RNA Template	x µl	Variable (0.1 pg - 1 µg)
Fluorogenic Probe (10µM)	0.2 µl	100nM (Variable 100-250 nM)
Volume with distilled water	to 20 µl	

Note: *Reaction volume can be scaled from 5 - 50 µl depending on the reaction plate (i.e. 384-well vs. 96-well) and qRT-PCR kit. Scale all component volumes proportionally.*

3. After capping each reaction, vortex gently to mix contents, centrifuge briefly to collect components at the bottom of the reaction tube. The assembled reactions should be kept cold to maximize the sensitivity and specificity of the assay until placed in real-time PCR system.

4. Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification.

	Standard Cycling	Fast Cycling
cDNA Synthesis	50°C, 10 min	50°C, 5 min
RT inactivation & Taq activation	95°C, 2 min	95°C, 2 min
PCR cycling (30-45 cycles)	95°C, 10-15s	95°C, 3-5s
	60°C, 30-60s	60°C, 20-30s
	(Data collection)	(Data collection)

Optimal cycling conditions will vary for different primer sets.
Melting curve analysis (Refer to instrument documentation).

Note: *Efficient cDNA synthesis can be achieved between 3-15 minutes of incubation at 45-55°C. We recommend 5 minutes of incubation at 50°C as a general starting point.*

5. Place reactions in a preheated real-time instrument programmed as described above. Collect data and analyze results.

Recommendations and Guidelines for One Step qRT-PCR

Instrument Compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the Bio-Rad CFX96 Touch, CFX384 Touch, CFX Connect, the Corbett Research Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; Illumina Eco qPCR…etc.

Reagent	Catalog Number	Compatible Real-Time PCR System
Biomiga One-Step qRT-PCR System	QP1318	Bio-Rad CFX96, CFX384, CFX Connect; the Corbett Research Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; Illumina Eco qPCR; Roche LightCycler™ 480; Eppendorf Mastercycler® ep realplex
Biomiga One-Step qRT-PCR System, Low Rox	QP1319	ABI® 7500, 7500 Fast, ViiA 7 Real-Time PCR Systems, the Stratagene Mx3000P®, Mx3005P™, and Mx4000®; the Agilent AriaMx
Biomiga One-Step qRT-PCR System, Rox	QP1320	ABI® 7000, 7300, 7700, 7900, 7900HT, StepOne™, StepOnePlus ™ Real-Time PCR Systems

Template

Starting material can range from 1 pg to 1 µg of purified total RNA. RNA should be free of RNase contamination and aseptic conditions should be maintained. RNA may be treated with DNase I to remove any contaminating DNA.

Magnesium Concentration

The 2X Reaction Mix includes magnesium at a final concentration of 3 mM. This works well for most targets; however, the optimal concentration may range from 3 to 6 mM. If necessary, use the following table to determine the amount of $MgCl_2$ to add to achieve the specified concentration:

For a Final $MgCl_2$ Concentration of	Add this Volume of 50 mM $MgCl_2$ (per 50 µl Rxn)
4.0 mM	1 µl
5.0 mM	2 µl
6.0 mM	3 µl

Melting Curve Analysis

Melting curve analysis should always be performed during qRT-PCR to identify the presence of primer dimers and analyze the specificity of the reaction.

Primers

Gene-specific primers are required. The length of amplicon should be approximately 70–150 bp, and the primers should be designed to anneal to exons on both sides of an intron or within the exon/exon boundary of the mRNA to allow differentiation of cDNA from genomic DNA. A final concentration of 200 nM per primer is effective for most reactions. Optimal results may require a titration of primer concentrations between 200 and 900 nM.

Troubleshooting Guide

Problem	Possible Cause	Solution
Poor sensitivity	Not enough template RNA	Increase concentration of template RNA; use 10 ng–1 µg total RNA.
Product detected at higher than expected cycle number	RNA has been damaged/degraded RNase contamination Inefficient cDNA synthesis Inefficient PCR amplification	Replace RNA if necessary. Maintain aseptic conditions; add RNase inhibitor. Adjust cDNA synthesis temperature and/or primer design. Double the amount of reverse primer (*e.g.,* to 400 nM). Optimize PCR conditions: Adjust annealing temperature as necessary. Increase magnesium concentration. Redesign primers.
Higher than expected signal	Too much sample added to reactions	Decrease the concentration of template RNA.
Product detected at lower-than-expected cycle number, and/or positive signal from no-template controls	Template or PCR carry-over contamination	Isolate source of contamination and replace reagent(s). Use separate dedicated pipettors for reaction assembly and post-PCR analysis. Assemble reactions (except for target addition) in a DNA-free area. Use aerosol-resistant pipet tips or positive displacement pipettors.
Unexpected bands after electrophoresis	Genomic DNA contamination Oligo(dT) or random primers used for cDNA synthesis	Pre-treat RNA with DNase I. Use only gene-specific primers.