



The Inventor of EZgene™ and ViraTrap™ Systems

## Biomiga™ Probe-Based 2× RT-qPCR Master Mix (QP1319)

Instruction for Use (IFU) v2600302

### 1. Intended Use and Product Description

Biomiga™ Probe-Based 2× RT-qPCR Master Mix (QP1319) is a single-tube, probe-based reverse transcription quantitative PCR (RT-qPCR) reagent designed for one-step detection and quantification of RNA targets. The reagent is suitable for both singleplex and multiplex assays and is compatible with research and molecular diagnostic workflows.

Reverse transcription and real-time PCR amplification are performed sequentially within the same reaction tube, simplifying workflow, reducing handling steps, and minimizing the risk of cross-contamination.

The formulation incorporates an antibody-mediated hot-start DNA polymerase, an optimized reverse transcriptase, and a proprietary reaction buffer system engineered for fully premixed RT-qPCR applications. Primers and probes may be pre-mixed with the master mix; users only need to add RNA templates during assay setup, improving operational efficiency and reducing reagent loss.

The mix includes a dUTP/UDG carryover prevention system that remains active at room temperature, degrading uracil-containing contaminant amplicons prior to amplification and significantly reducing aerosol-mediated PCR carryover contamination.

### 2. Features and Advantages

- Primer and probe premixing supported with stability demonstrated at 37 °C for up to 100 hours.
- Fast RT-qPCR protocols enabling results in approximately 40 minutes.
- High sensitivity and specificity enabled by genetically engineered antibody-based hot-start Taq polymerase.
- Integrated dUTP/UDG anti-contamination system to prevent carryover amplification.
- Broad instrument compatibility including QuantStudio™ 5/7 Flex/12K, ABI 7500, Bio-Rad CFX96, Roche LightCycler® 480 II / LightCycler® 96 / LightCycler® PRO, and other standard real-time PCR platforms

### 3. Components

This ready-to-use one tube 2× master mix.

Cat#	Description	Size
QP1319-01	2x RT-qPCR Mix, 100 reactions	1 mL
QP1319-02	2x RT-qPCR Mix, 500 reactions	5 mL
QP1319-03	2xRT-qPCR Mix, 1000 reactions	10 mL



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- Reverse Transcriptase
- Hot-Start Taq DNA Polymerase
- dNTPs (including dUTP)
- Reaction stabilizers and enhancers
- Mg<sup>2+</sup>
- UDG (Uracil-DNA Glycosylase) system

The dUTP/UDG system removes carryover contamination by degrading uracil-containing DNA prior to amplification, reducing false-positive results and improving assay reliability.

#### 4. Storage and Stability

- Store at -20 °C.
- Avoid repeated freeze-thaw cycles.
- Protect from prolonged light exposure.
- Thaw on ice and mix gently before use.
- When stored properly, the product is stable until the expiration date indicated on the label (12 months from the date of purchase).

#### 5. Reaction Setup (20 µL Total Volume)

Prepare reactions on ice in a clean PCR setup area.

Component	Volume per Reaction
2× RT-qPCR Master Mix	10 µL
RNA Sample / Positive Control / Negative Control + Nuclease-Free Water	10 µL
Total Volume	20 µL

- Mix gently and briefly centrifuge before loading into the instrument.

#### 6. Recommended Thermal Cycling Program

Step	Temperature	Time	Cycles
UDG Activation (Contamination Removal)	37 °C	2 min	1
Reverse Transcription	50 °C	5 min	1
Initial Denaturation	95 °C	1 min	1



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Denaturation	95 °C	5 sec	40
Annealing/Extension*	60 °C	15 sec	40

\*Annealing/extension temperature may be optimized depending on primer/probe design.

## 7. Quality Control Recommendations

- Include no-template control (NTC) to monitor contamination.
- Include positive control to verify assay performance.
- Use an internal control to monitor extraction and amplification efficiency when applicable.

## 8. Precautions and Limitations

- Use RNase-free consumables and reagents.
- Prepare reactions in a clean environment separate from amplification areas.
- Avoid repeated freeze-thaw cycles.
- Performance depends on primer/probe design and sample quality.
- For research use or laboratory testing workflows unless otherwise validated by the user.

## 9. Manufacturer Information

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