



**PCR BIOSYSTEMS**

simplifying research

**qPCR BIO Probe**

**1-Step Go No-ROX**

[www.pcrbio.com](http://www.pcrbio.com)

## Product description:

qPCR BIO Probe 1-Step Go is a universal probe kit designed for fast, highly specific and ultra-sensitive RT-qPCR. The latest developments in reverse transcriptase technology and buffer chemistry are used to give efficient cDNA synthesis and real-time PCR in a single tube.

The kit is engineered for use on a wide range of probe technologies such as TaqMan®, Scorpions® and molecular beacon probes. It can be used to quantify any RNA template including mRNA, total RNA and viral RNA sequences. qPCR BIO Probe 1-Step Go is designed to give rapid and accurate results over a broad range of template concentrations and is ideally suited to the detection of RNA viruses including SARS-CoV-2.

The kit includes a thermostable and extremely active modified MMLV reverse transcriptase (RTase Go) and advanced RNase inhibitor that prevents degradation of RNA by contaminating RNase. Antibody-mediated hot start technology prevents the formation of primer dimers and non-specific products giving highly specific and ultra-sensitive real-time RT-PCR with unrivalled efficiency in multiplex.

High-throughput screening has resulted in a buffer system that allows efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions.

| Component                              | 100 rxns  | 300 rxns  | 1200 rxns  |
|----------------------------------------|-----------|-----------|------------|
| 2x qPCR BIO Probe<br>1-Step Go No-ROX  | 1 x 1mL   | 3 x 1mL   | 12 x 1mL   |
| 20x RTase Go<br>(with RNase inhibitor) | 1 x 100µL | 3 x 100µL | 12 x 100µL |

## Shipping and storage

On arrival the kit should be stored between -30°C and -15°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

## Limitations of product use

The product may be used only for in vitro research purposes.

## Technical support

Help is available on our website at <https://pcrbio.com/resources/> including answers to frequently asked technical questions. For technical support and troubleshooting please email [technical@pcrbio.com](mailto:technical@pcrbio.com) with the following information:

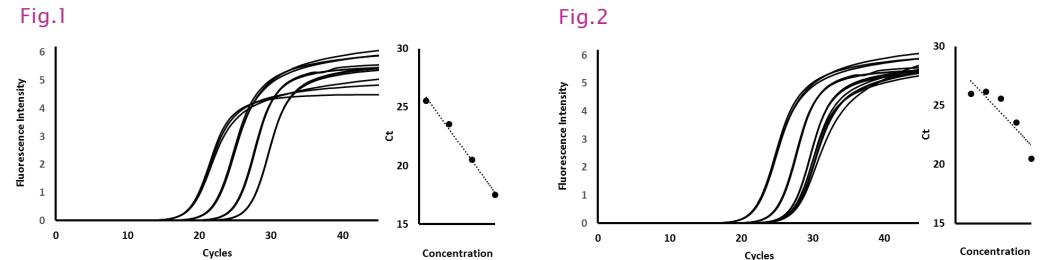
- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile

## Important considerations

**Instrument compatibility:** Different real-time PCR instruments require different levels of ROX passive reference. Generally, modern instruments do not require passive reference but include the option to use it for normalisation. Please check our qPCRBIO Selection Table to determine which ROX concentration your instrument requires (<https://pcrbio.com/resources/qpcr-selection-tool/>).

**Primer design:** For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80bp and 200bp. With all manufacturers, the shorter the amplicon length, the faster the reaction can be cycled. Amplicon lengths should not exceed 400bp. Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (<http://bioinfo.ut.ee/primer3/>). For TaqMan® probes, choose a probe close to the 5' primer and avoid terminal guanosine residues.

**Template concentration:** As target copy number will vary, it is important to select the correct template concentration to correctly quantify the target sequence. A good concentration will display clear separation between amplification curves (Fig.1). At lower template concentrations, the amplification curves will begin to group together and Ct values will not fit the standard curve (Fig.2).



## Reaction setup

1. Before starting, briefly vortex 2x qPCRBIO Probe 1-Step Go Mix.
2. Prepare a master mix based on the following table. We also recommend setting up a no-RTase control:

| Reagent                        | 20µL reaction                                                                       | Final conc. | Notes                                                                                                                                  |
|--------------------------------|-------------------------------------------------------------------------------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------|
| 2x qPCRBIO Probe 1-Step Go Mix | 10µL                                                                                | 1x          | See above for optimal primer design                                                                                                    |
| Forward primer (10µM)          | 0.8µL                                                                               | 400nM       |                                                                                                                                        |
| Reverse primer (10µM)          | 0.8µL                                                                               | 400nM       |                                                                                                                                        |
| Probe (10µM)                   | 0.4µL                                                                               | 200nM       |                                                                                                                                        |
| 20x RTase Go                   | 1.0-2.0µL                                                                           | 1x or 2x    | 1.0µL is recommended. 2.0µL will improve Ct but may increase primer-dimers                                                             |
| Template RNA                   | Viral RNA: 10 to 1x10 <sup>8</sup> copies<br>Total RNA: 1pg to 1µg<br>mRNA: >0.01pg | Variable    | Addition of sample as 2 to 5µL volumes will improve assay precision. 5µL of swab extract is recommended for COVID-19 diagnostic assays |
| PCR grade dH <sub>2</sub> O    | Up to 20µL final volume                                                             |             |                                                                                                                                        |

3. Program the instrument using the following conditions, acquiring data on the appropriate channel:

| Cycles        | Temperature                      | Time                       | Notes                                                                                                                                                 |
|---------------|----------------------------------|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1             | 45°C to 55°C                     | 10min                      | Reverse transcription: 45°C is recommended for most applications. 55°C should be used only when amplicon contains regions of high secondary structure |
| 1             | 95°C                             | 2min                       | Polymerase activation                                                                                                                                 |
| 40            | 95°C<br>60°C to 65°C             | 5 seconds<br>20-30 seconds | Denaturation<br>Anneal/Extension: do not exceed 30 seconds, do not use temperatures below 60°C                                                        |
| Melt analysis | Refer to instrument instructions |                            | Optional melt profile analysis, available for hybridisation probes only                                                                               |