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qPCRBIO Probe 1-Step Go Separate-ROX

Product description

qPCRBIO 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. qPCRBIO 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.

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 go through 30 freeze/thaw cycles with no loss of activity.

Limitations of product use

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

Component	100 rxns	300 rxns	1200 rxns
2x qPCRBIO Probe 1-Step Go No-ROX	1 x 1mL	3 x 1mL	12 x 1mL
50µM ROX Additive	1 x 200µL	1 x 200µL	4 x 200µL
20x RTase Go (with RNase inhibitor)	1 x 100µL	1 x 300µL	4 x 300uL

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 with the following information:

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

Important considerations

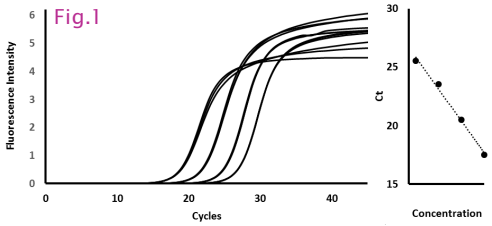
Instrument compatibility: Different real-time PCR instruments require different levels of ROX passive reference. Generally, modern instruments do not require passive reference but include the option to use it for normalisation. Please use our qPCRBIO Selection Tool to determine which ROX concentration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

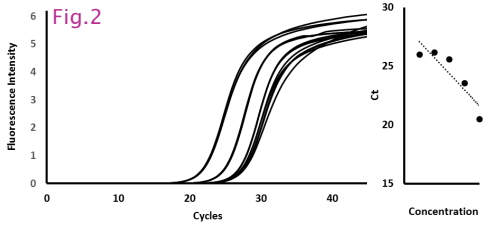
Primer design: For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80bp and 200bp. With all manufacturers' master mixes, the shorter the amplicon length, the faster the reaction can be cycled. Amplicon lengths should not exceed 400bp.

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.

Important considerations continued

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).





ROX additive protocol: The 50µM ROX Additive supplied is formulated to be added directly to the 1mL tube of 2x qPCRBIO master mix supplied. Once the ROX is added, the reagent may be used straight away or stored between -30˚C and -15˚C for future use. Please use the following 2 charts to add the correct amount of ROX for your instrument. Vortex thoroughly after ROX addition.

Hi-ROX instruments	Reagent volume	Final concentration	Reaction concentration
2x qPCRBIO Probe 1-Step Go No-ROX	1.0mL	2x	1x
50µM ROX Additive	20.0µL	1µM	500nM

Lo-ROX instruments	Reagent volume	Final concentration	Reaction concentration
2x qPCRBIO Probe 1-Step Go No-ROX	1.0mL	2x	1x
50µM ROX Additive	2.0µL	100nM	50nM

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 PCRBIO Probe 1-Step Go Mix	10µL	1x	
Forward primer (10µM)	0.8µL	400nM	See above for optimal primer design
Reverse primer (10µM)	0.8µL	400nM	
Probe (10µM)	0.4µL	200nM	
20x RTase Go	0.2µL	0.2x	0.2µL for sensitive SARS-CoV-2 detection. Alternatively, titrate down to 0.05uL.
Template RNA	Viral RNA: 10 to 1x10^8 copies Total RNA: 1pg to 1µg mRNA: >0.01pg	Variable	Addition of sample as 2 to 5µL volumes will improve assay precision. 5uL of swab extract is recommended for SARS-CoV-2 diagnostic assays
PCR grade dH_2O	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 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 60˚C to 65˚C	5 seconds 20-30 seconds	Denaturation Anneal/Extension: do not exceed 30 seconds, do not use temps below 60˚C
Melt analysis	Refer to instrument instructions		Optional melt profile analysis, available for hybridisation probes only