PCRBIOSYSTEMS
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PCRBIO 1-Step Go RT-PCR Kit

Product description:

PCRBIO 1-Step Go RT-PCR Kit is a convenient, easy-to-use kit for fast and efficient cDNA synthesis and PCR in a single tube. The advanced buffer system, reverse transcriptase and hot start polymerase give highly specific and ultra-sensitive 1-step RT-PCR from any RNA template.

The kit includes our modified thermostable reverse transcriptase (RTase Go) blended with an advanced RNase inhibitor to prevent degradation of RNA by contaminating RNase. The RTase is not inhibited by ribosomal and transfer RNAs making total RNA an ideal substrate.

Antibody-mediated hot start technology prevents the formation of primer dimers and non-specific amplification giving robust RT-PCR performance with minimal or no optimisation required.

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

Component	50 reactions	100 reactions	500 reactions
2x PCRBIO 1-Step Go Mix	1 x 1.25 mL	2 x 1.25 mL	10 x 1.25 mL
20x RTase Go with RNase inhibitor	1 x 125 µL	2 x 125 µL	10 x 125 µL

Shipping and storage

On arrival the kit should be stored between -30 °C and -15 °C. If stored correctly the kit will retain full activity for 12 months. Do not store the mix once it is combined with the RTase.

Limitations of product use

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

Technical support

Help and support 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
- Agarose gel images

Important considerations

2x PCRBIO 1-Step Go Mix: The 2x mix contains PCRBIO HS Taq DNA Polymerase, 6 mM $MgCl_2$, 2 mM dNTPs, enhancers and stabilizers. It is not recommended to add further PCR enhancers or $MgCl_2$ to the reaction. The buffer composition has been optimised to maximise PCR success rates.

20x RTase Go: The 20x RTase Go also contains RNase inhibitor. For difficult templates the yield of reaction can be increased by reducing the amount of RTase Go added. In this case we recommend a titration (0.2x - 1x).

Template: 1 pg to 1 µg of total RNA are recommended for accurate quantification. Up to 5 µg of total RNA may be added for increased cDNA yield, however complete reverse transcription of these high amounts is not guaranteed. For mRNA, use a minimum of 0.01pg per reaction.

Primers: Primers should have a predicted melting temperature of around 60 ˚C, using default Primer 3 settings (http://bioinfo.ut.ee/primer3/). The final primer concentration in the reaction should be between 0.2 µM and 0.6 µM.

Reverse Transcription: We recommend incubating with a temperature of 45 ˚C for 10-20 minutes for the majority of applications. Where regions of interest contain high secondary structure incubation temperatures up to 55 ˚C may be used. For amplicons above 1 kb the incubation time may be increased.

Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 55 ˚C annealing temperature then increase in 2 ˚C increments if non-specific products are present.

Extension: Optimal extension is achieved at 72 ˚C. The optimal extension time is dependent on amplicon length and complexity of template. 15 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1 kb and 3 kb.

Reaction setup

1. Before starting, briefly vortex 2x PCRBIO 1-Step Go Mix

2. Prepare a master mix based on following table. We recommend also setting up a no-RTase control:

Reagent	50 µL reaction	Final concentration	Notes
2x PCRBIO 1-Step Go Mix	25 µL	1x	
Forward primer (10 µM)	2.0 µL	400 nM	See above for optimal primer design
Reverse primer (10 µM)	2.0 µL	400 nM	
20x RTase Go	2.5 µL	1x	Lower volumes can be used for difficult templates (see important considerations)
Template RNA	1 pg to 1 µg total RNA >0.01 pg mRNA	variable	
PCR grade dH_2O	Up to 50 µL final volume		

3. Program the instrument using following conditions:

Cycles	Temperature	Time	Notes
1	45 ˚C to 55 ˚C	10-20 minutes	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	2 minutes	Polymerase activation
40	95 ˚C 55 ˚C to 65 ˚C 72 ˚C	10 seconds 10 seconds 30-60 seconds	Denaturation Anneal 15 seconds per kb