PCRBIOSYSTEMS
simplifying research

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 1.25ml	2 x 1.25ml	10 x 1.25ml
20x RTase Go with RNase inhibitor	1 x 125µl	2 x 125µl	10 x 125µl

Shipping and storage

On arrival the kit should be stored at -20˚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

For technical support and troubleshooting please email technical@pcrbio.com 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, 6mM $MgCl_2$, 2mM 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. It is essential to use the correct volume per reaction. Using the incorrect volume will result in loss of sensitivity.

Template: 1pg to 1µg of total RNA are recommended for accurate quantification. Up to 5µg 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://frodo.wi.mit.edu/primer3/). The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

Reverse Transcription: We recommend incubating with a temperature of 45˚C for 10 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 1kb the incubation time should be increased to 20 minutes.

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 1kb and 3kb.

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	50µl reaction	Final concentration	Notes
2x PCRBIO 1-Step Go Mix	25µl	1x	
Forward primer (10µM)	2.0µl	400nM	See above for optimal primer design
Reverse primer (10µM)	2.0µl	400nM	
20x RTase Go	2.5µl	1x	Correct volume is critical, do no reduce or increase
Template RNA	1pg to 1µg total RNA >0.01pg mRNA	variable	
PCR grade dH_2O	Up to 50µl final volume		

3. Program the instrument using following conditions:

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 60˚C to 65˚C 72˚C	10 seconds 10 seconds 30-60 seconds	Denaturation Anneal 15 seconds per kb