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PCRBIO 1-Step Go RT-PCR Kit Red

Product description

PCRBIO 1-Step Go RT-PCR Kit Red is a convenient, easy-to-use kit for fast and efficient cDNA synthesis and PCR in a single tube, with the convenience of direct loading onto agarose gels without the need for additional loading buffer. 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 RNases. 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.

This kit is suitable for classical RT-PCR experiments and is ideal for investigating transcript splice variants, investigating RNAi effects, and detection of full-length transcripts

Quality control

PCRBIO Systems operates under an ISO 13485 certified Quality Management System. Our products are extensively tested and undergo a comprehensive, multi-step quality control process according to ISO 13485 standards, to ensure optimum performance, consistency and traceability.

Product Use: Unless we agree otherwise in writing, the Goods we supply are provided:

1. For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
2. Subject to our standard terms and conditions found at <https://pcrbio.com/terms-conditions/>.

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

Shipping and storage

On arrival the kit should be stored between -30 °C and -20 °C. If stored correctly, the kit will retain full activity until the indicated expiry date. Avoid exposure of the stock solution to frequent temperature changes and limit handling at room temperature to the necessary minimum. Do not store the mix once it is combined with the RTase.

Technical support

Scan or click the QR code for our primer Tm calculator and answers to frequently asked technical questions. For further technical support, please email technical@pcrbio.com with the following information:

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



TM CALCULATOR



FAQS

Important considerations

2x PCR BIO 1-Step Go Mix Red: The 2x mix contains PCR BIO HS Taq DNA Polymerase, 6 mM MgCl₂, 2 mM dNTPs, enhancers, stabilizers, and a red dye for tracking during agarose electrophoresis. Further addition of PCR enhancers or MgCl₂ to the reaction is not recommended. The buffer's 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 0.01 pg per reaction.

Primer design: Primers should have an approximate T_m of ~60 °C using default Primer 3 settings (<https://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: To verify the best annealing temperature for your primers in our products, please visit <https://pcrbio.com/resources/tm-calculator/>. If troubleshooting indicates issues with primer 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 PCR BIO 1-Step Go Mix Red
2. Prepare a master mix based on the following table. We recommend setting up a no-RTase control:

Reagent	50 µL reaction	Final concentration	Notes
2x PCR BIO 1-Step Go Mix Red	25 µL	1x	
Forward primer (10 µM)	2 µL	400 nM	See above for optimal primer design
Reverse primer (10 µM)	2 µL	400 nM	
20x RTase Go	2.5 µL	1x	See notes above
Template DNA	1 pg to 1 µg total RNA >0.01 pg mRNA	variable	See "Template" above
PCR grade dH ₂ O	Up to 50 µL final volume		

3. Cycle using conditions based on the following table:

Cycles	Temperature	Time	Notes
1	45 °C - 55 °C	10-20 minutes	Reverse transcription: 45 °C is recommended for most applications. 55 °C should be used only when amplicons contain regions of high secondary structure or high GC content (> 60%)
1	95 °C	2 minutes	Polymerase activation
40	95 °C	10 seconds	Denaturation
	55 °C - 65 °C	10 seconds	Anneal
	72 °C	15 s/kb	Extension (see notes above)