PCRBIOSYSTEMS
simplifying research

# PCRBIO One-Step RT-PCR Kit

## Product description:

PCR Biosystems PCRBIO One-Step RT-PCR Kit uses the latest developments in reverse transcriptase technology and buffer chemistry for efficient cDNA synthesis and PCR in a single tube.

Our modified MMLV reverse transcriptase (RTase) is both thermostable and extremely active. The enzyme is blended with RNase inhibitor preventing degradation of RNA by contaminating RNase. The RTase is not inhibited by ribosomal and transfer RNAs, total RNA is an ideal substrate.

PCRBIO One-Step RT-PCR Kit uses proprietary small molecular inhibitor technology that prevents formation of primer-dimers to improve reaction sensitivity and specificity.

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 | 50 reactions | 100 reactions | 500 reactions |
|---|---|---|---|
| 2x PCRBIO One-Step Mix | 1x 1.25ml | 2x 1.25ml | 10x 1.25ml |
| 20x RTase with RNase inhibitor | 1x 125µl | 2x 125µl | 10x 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 One-Step 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: The 20x RTase 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: Use 1pg to 1µg total RNA per reaction, use minimum 0.01pg mRNA 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 One-Step 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 One-Step Mix | 25µl | 1x | |
| Forward primer (10µM) | 2.0µl | 400nM | See above for optimal primer design |
| Reverse primer (10µM) | 2.0µl | 400nM | |
| 20x RTase | 2.5µl | 1x | Correct volume is critical, do no reduce |
| Template RNA | 1pg to 1µg total RNA<br>>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, 2 minutes |
| 40 | 95˚C<br>60˚C to 65˚C<br>72˚C | 10 seconds<br>10 seconds<br>30-60 seconds | Denaturation<br>Anneal<br>15 seconds per kb |