PCRBIOSYSTEMS
simplifying research

qPCRBIO Probe 1-Step Hi-ROX

Product description:

PCR Biosystems qPCRBIO Probe 1-Step 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 making total RNA an ideal substrate.

PCR Biosystems real-time PCR probe mixes have been designed for use on a wide range of probe technologies including TaqMan®, Scorpions® and molecular beacon probes.

qPCRBIO Probe 1-Step Mix uses antibody-mediated hot start technology that prevents the 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	100 reactions	300 reactions
2x qPCRBIO Probe 1-Step	1 x 1ml	3 x 1ml
20x RTase with RNase inhibitor	1 x 200µl	3 x 200µl

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
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 check our qPCRBIO Selection Table to determine which ROX concentration your instrument requires (http://www.pcrbio.com/realtime-pcr.html).

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://frodo.wi.mit.edu/primer3/). For TaqMan® probes, choose a probe close to the 5' primer and avoid terminal guanosine residues.

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.

Reaction setup

1. Before starting, briefly vortex 2x qPCRBIO Probe 1-Step Mix

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

Reagent	20µl reaction	Final concentration	Notes
2x qPCRBIO Probe 1-Step 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	1.0-2.0µl	1x or 2x	1.0µl is recommended. 2.0µl will improve Ct but may increase primer dimers
Template RNA	1pg to 1µg total RNA >0.01pg mRNA		
PCR grade dH_2O	Up to 20µl final volume		

3. Program the instrument using following conditions, acquiring data on the appropriate channel:

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	5 seconds 20-30 seconds	Denaturation Anneal/Extension: do not exceed 30 seconds, do not use temperatures below 60˚C
Melt analysis	Refer to instrument instructions		Optional melt profile analysis, available for hybridisation probes only