



PCRBIO SYSTEMS
simplifying research

qPCR BIO SyGreen 1-Step Detect Hi-ROX

www.pcrbio.com

Product description:

PCR Biosystems qPCR BIO SyGreen 1-Step Detect 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 SyGreen Mixes use an intercalating dye which does not inhibit PCR, unlike other popular dyes.

qPCR BIO SyGreen 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 rxns | 300 rxns | 1200 rxns |
|-----------------------------------|-----------|-----------|------------|
| 2x qPCR BIO SyGreen 1-Step Hi-ROX | 1 x 1ml | 3 x 1ml | 12 x 1ml |
| 20x RTase with RNase inhibitor | 1 x 200µl | 3 x 200µl | 12 x 200µl |

Shipping and storage

On arrival the kit should be stored between -30°C and -15°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 qPCR^{BIO} 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/>).

Template concentration: As target copy number will vary, it is important to select the correct template concentration to correctly quantify the target sequence. A good concentration will display separation between amplification curves (Fig.1). At higher template concentrations, the amplification curves will begin to group together and Ct values will not fit the standard curve (Fig.2).

qPCR^{BIO} SyGreen 1-Step Detect is engineered to give rapid and accurate results from low template concentrations. If you observe grouping at higher template concentrations, try diluting the template. Alternatively, try qPCR^{BIO} SyGreen 1-Step Go Lo-ROX Kit (PB25.31-03), which is engineered for higher concentrations.

Fig.1

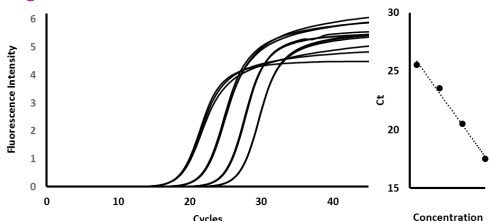
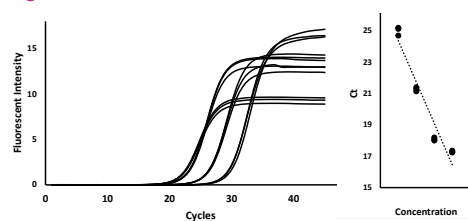


Fig.2



Reaction setup

1. Before starting, briefly vortex 2x qPCR^{BIO} SyGreen 1-Step Mix
2. Prepare a master mix based on the following table. We also recommend setting up a no-RTase control:

| Reagent | 20µl reaction | Final concentration | Notes |
|---|---------------------------------------|---------------------|---|
| 2x qPCR ^{BIO} SyGreen 1-Step Mix | 10µl | 1x | |
| Forward primer (10µM) | 0.8µl | 400nM | See above for optimal primer design |
| Reverse primer (10µM) | 0.8µl | 400nM | |
| 20x RTase Go (contains RNase inhibitor) | 1.0µl | 1x | Add before template |
| Template RNA | 1pg to 10ng total RNA >0.01pg mRNA | Variable | See above for optimal template amounts. Up to 5µg total RNA may be added for increased cDNA yield, however complete reverse transcription of these high amounts is not guaranteed |
| PCR grade dH ₂ O | Up to 20µl final volume | | |

3. Program the instrument using the 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 |