TECHNICAL DATA SHEET

CYBRFast™ 1-Step RT-qPCR Lo-ROX Kit

Catalog Numbers: 31-5201-0100R
31-5201-0300R

PRODUCT INFORMATION

Contents: 31-5201-0100R (100 rxns)
CYBRFast™ qPCR Lo-ROX Master Mix (2X): 1 x 1.0 mL
M-MLV RTase / RNase Inhibitor Mix (20X): 1 x 200 µL

31-5201-0300R (300 rxns)
CYBRFast™ qPCR Lo-ROX Master Mix (2X): 5 x 1.0 mL
M-MLV RTase / RNase Inhibitor Mix (20X): 3 x 200 µL

Use By: 6 months from date of receipt

DESCRIPTION

The CYBRFast™ 1-Step RT-qPCR Lo-ROX Kit's advanced reagents allow for efficient cDNA synthesis and qPCR in a single tube using real-time instruments that support normalization with a standard ROX passive reference dye at a final concentration of 50 nM. CYBRFast™ Hot Start Taq DNA Polymerase, a next generation thermostable polymerase of recombinant origin that possess 5'→3' polymerase activity, is included in the Master Mix (2X). It also includes two proprietary CYBRFast™ Hot Start mAbs (monoclonal antibodies) which are used to specifically block polymerase activity below 70°C, allowing for convenient room temperature reaction set up. Polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 94-95°C for two minutes. The blocking of polymerase activity prior to denaturation of template improves yields by minimizing or eliminating primer dimer formation and non-specific amplification. This hot start polymerase has been optimized for use with the CYBRFast™ GREEN fluorescent intercalating dye in real-time applications and reliably delivers excellent signal to noise ratios, rapid extension times for early Ct values and enhanced sensitivity. In addition, a proprietary 20X M-MLV Reverse Transcriptase (RT) molecule blended with an optimized RNase inhibitor is included in a separate tube. The RT enzyme can synthesize cDNA at a temperature range of 45–55°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Our M-MLV RT is not significantly inhibited by ribosomal and transfer RNA, making it ideal to synthesize cDNA from total RNA. Convenient and reliable, Tonbo's CYBRFast 1-Step RT-qPCR Lo-ROX Kit is ideal for the sensitive and efficient quantification of RNA templates, including low copy number targets, under standard or fast cycling conditions.

RT-PCR (reverse transcription-polymerase chain reaction) is used to convert and amplify a single-stranded RNA template to yield abundant double-stranded complementary DNA (cDNA) product. One-step RT-PCR is a variation on standard RT-PCR in which all components are mixed in one tube prior to starting the reactions. This approach offers simplicity and convenience and minimizes the possibility for contamination.

STORAGE

Store reagent at -20°C upon arrival and limit exposure to light. This product may undergo up to 30 freeze/thaw cycles without loss of activity. When stored correctly this product will retain activity for up to 6 months. This kit can be stored at 4°C for up to 1 month.

BIOLOGICAL SOURCE

The CYBRFast Hot Start Taq DNA Polymerase enzyme is a single recombinant polypeptide of bacterial origin having a molecular weight of ~94 kDa originally derived from the YT-1 strain of *Thermus acquaticus*. CYBRFast Hot Start mAbs are of murine origin and are reactive with select epitopes found within recombinant forms of the YT-1 strain of *Thermus acquaticus*. Tonbo's Reverse Transcriptase is a proprietary version of M-MLV RT that has been genetically engineered to reduce RNase H activity and provide increased thermal stability.

INSTRUMENT COMPATIBILITY

Tonbo's CYBRFast 1-Step RT-qPCR Lo-ROX Kit can be used on a wide variety of real-time instruments including those that do not have a ROX channel. Please Note: This Kit is NOT compatible with instruments that require a high concentration ROX dye. We offer our CYBRFast 1-Step RT-qPCR Hi-ROX Kit (Cat. No. 31-5202) for use with those instruments. Please see the Real-Time PCR Instrument Compatibility Guide (pg. 3) for additional information.

APPLICATION NOTES

Master Mix: CYBRFast 1-Step Lo-ROX Master Mix (2X) contains CYBRFast Hot Start *Taq* DNA Polymerase, CYBRFast Hot Start mAbs, CYBRFast GREEN fluorescent dye, 6 mM $MgCl_2$, 2 mM dNTPs and a low concentration ROX reference dye in a buffer that includes a proprietary mix of stabilizers and enhancers. This Master Mix has been rigorously developed for optimal PCR success rate, yield and efficiency. We do not recommend introducing additional $MgCl_2$ or enhancers to the reaction mix.

M-MLV RTase / RNase Inhibitor Mix (20X): Use 1-2 µL per reaction - 1 µL is recommended. While 2 µL may improve C_t, it may also increase primer-dimer formation. Please refer to the reaction mix preparation step under Reaction Setup/Quick Protocol.

ROX: 50 nM final concentration. Carboxy-X-Rhodamine (ROX) is an inert passive reference dye used in real-time PCR reactions. Non-PCR related fluctuations in fluorescence are most often attributed to well-to-well optical path length variations, but may also come from bubble formation during the PCR reaction, pipetting errors or poor mixing. Because it does not interfere with the PCR reaction and the level of fluorescence will not change during the reaction, ROX can be used to generate a baseline fluorescence that is used to normalize for signal intensity. As instruments have different optical configurations, different optimal concentrations of the ROX dye are required. Please refer to our Instrument Compatibility Guide to ensure you have the correct ROX concentration for your system.

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Primers: HPLC-purified primers are recommended. We suggest primers have a predicted melting temperature of approximately 60°C using default Primer 3 settings (http://bioinfo.ut.ee/primer3/). For each reaction, a final primer concentration of 0.2 - 06 µM is suggested. Amplicon lengths between 80 - 200 bp, and not larger than 400 bp, are recommended for efficient amplification. Shorter amplicons allow for faster cycling.

Template: We recommend using between 1 pg - 1 µg of total RNA per reaction. For mRNA, use at least 0.01 pg per reaction.

Reverse Transcription Reaction: Incubation for 10 minutes at 45°C should work well for most applications, however one may consider the following adjustments as appropriate. Increase the incubation time to 20 minutes for amplicons larger than 1 kilobase (kb). Temperature can be increased up to 55°C in cases where regions of interest have a high degree of secondary structure (>65% GC).

Annealing/Extension: For this reaction, we recommend an annealing/extension temperature between 60-65°C. Do not use temperatures below 60°C. Do not exceed 30 seconds per cycle.

Melt Curve Analysis: While optional, we recommend performing a melt curve analysis in order to analyze the specificity of the reaction and to be able to identify the presence of any primer-dimers. Please refer to the manufacturer's instructions provided with your instrument.

REACTION SETUP / QUICK PROTOCOL

1. Ensure all components are thawed and mixed well. Set up reactions on ice. Refer to the following table for reaction preparation. If preparing multiple reactions, assemble all common components into a master reaction mix. If working with final reaction volumes greater than 20 µl, scale component volumes accordingly.
2. Optional: We recommend including a no-RTase control reaction (do not add M-MLV RTase / RNase Inhibitor Mix) which will act as a control for the presence of contaminating genomic DNA in the reaction. A no-template control (do not add template RNA) can also be set up as a control for the presence of contaminating genomic DNA in the enzyme/primer mixes.
3. As applicable, transfer the recommended volume of master reaction mix, primers and sample template RNA to individual PCR tubes or plates, seal and spin briefly to mix.
4. Refer to the cycling conditions below to perform the reaction. Acquire data in the SYBR Green or FAM channel.

Table 1. Reaction Preparation

Reagent	20 µL reaction	Final Concentration	Notes
CYBRFast 1-Step Master Mix (2X)	10.0 µL	1x	
Forward Primer (10 µM)	0.8 µL	400 nM	See above for optimal primer design
Reverse Primer (10 µM)	0.8 µL	400 nM	
20X Rtase	1.0-2.0 µL	1x or 2x	1.0 µL is recommended 2.0 µL will improve C_t but may increase primer dimers
Template RNA	1 pg to 1µg total RNA >0.01 pg mRNA	variable	See above for template considerations
Nuclease free dH_2O	Up to 20 µL final volume		

Table 2. Cycling Conditions

Cycles	Temperature	Time	Notes
1	45°C - 55°C	10 minutes	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	2 minutes	Polymerase activation, 2 minutes
	95°C	5 seconds	Denaturation
40	60°C - 65°C	20-30 seconds	Anneal/Extension, do not exceed 30 seconds; do not use tempuratures below 60°C
Melt Analysis	Refer to instrument instructions		Optimal melt profile analysis

TECHNICAL SUPPORT

Please provide the following information to support@tonbobio.com for troubleshooting and technical support:

- Catalog and batch numbers
- Reaction set-up (master mix)
- Cycling conditions
- Amplicon size
- Screen shots of amplification traces and melting profile
- Detailed description of the issue.

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