TECHNICAL DATA SHEET

FastPROBE™ 1-Step RT-qPCR Hi-ROX Kit

Catalog Numbers: 31-5212-0100R
31-5212-0300R

PRODUCT INFORMATION

Contents: 31-5212-0100R (100 rxns)
FastPROBE™ 1-Step Hi-ROX Master Mix (2X): 1 x 1.0 mL
M-MLV RTase / RNase Inhibitor Mix (20X): 1 x 200 µL

31-5212-0300R (300 rxns)
FastPROBE™ 1-Step Hi-ROX Master Mix (2X): 3 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 FastPROBE™ 1-Step RT-qPCR Hi-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 500 nM. Classic++™ 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 Classic++™ Hot Start mAbs (monoclonal antibodies) which are used to specifically block polymerase activity below 70°C, allowing for convenient room temperature reaction set up. DNA 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 template denaturation improves yields by minimizing or eliminating primer dimer formation and non-specific amplification. Classic++ Hot Start *Taq* polymerase is compatible with all fluorogenic probes, including hydrolysis and displacement probes. In addition, a proprietary 20X M-MLV Reverse Transcriptase (RT) molecule blended with an optimized RNase inhibitor is included in a separate tube. The 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.

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.

Convenient and reliable, Tonbo's FastPROBE 1-Step RT-qPCR Hi-ROX Kit is ideal for both standard and fast-cycling real-time RT-PCR applications using sequence-specific fluorogenic probes. It reliably delivers excellent signal to noise ratios, increased speed and linearity, and enhanced sensitivity. The FastPROBE 1-Step RT-qPCR Hi-ROX Kit can be used for the absolute quantitation of gene expression from a variety of RNA templates and is ideal for low copy number detection, microarray validation, SNP genotyping and multiplexing.

STORAGE

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

BIOLOGICAL SOURCE

The Classic++ 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*. Classic++ 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 FastPROBE 1-Step RT-qPCR Hi-ROX Kit has been optimized for use with real-time instruments requiring a high concentration of ROX dye. **Please Note**: This Kit is NOT compatible with instruments that require a low concentration ROX dye. We offer our FastPROBE 1-Step RT-qPCR Lo-ROX Kit (Cat. No. 31-5211) for use with those instruments. We also provide a No-ROX version, Cat. No. 31-5210. Please see the Real-Time PCR Instrument Compatibility Guide (pg. 3) for additional information.

APPLICATION NOTES

Master Mix: FastPROBE 1-Step Hi-ROX Master Mix (2X) contains Classic++ Hot Start *Taq* DNA Polymerase, Classic++ Hot Start mAbs, 6 mM $MgCl_2$, 2 mM dNTPs and a high 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: 500 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 (pg. 3) 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 TaqMan® probes avoid terminal quanosine residues and choose a probe close to the 5' primer. 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. Refer to Table 1 for reaction preparation. If preparing multiple reactions, assemble all common components into a master mix. If working with final reaction volumes less 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 mix, primers and sample template RNA to individual PCR tubes or plates, seal and spin briefly to mix. Refer to the cycling conditions (Table 2) to perform the PCR. Acquire data on the channel appropriate to the probe reporter dye.

Table 1. Reaction Preparation

Reagent	20 µL reaction	Final Concentration	Notes
FastPROBE 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	
Probe (10 µM)	0.4 µL	200 nM	
20X RTase Mix	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
	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; available for hybridization probes only.

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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