TECHNICAL DATA SHEET

FastPROBE™ qPCR No-ROX Master Mix

Catalog Numbers: 31-5110-0100R
31-5110-0500R

PRODUCT INFORMATION

Contents: 31-5110-0100R (100 rxns)
FastPROBE™ qPCR No-ROX Master Mix (2X): 1 x 1.0 mL

31-5110-0500R (500 rxns)
FastPROBE™ qPCR No-ROX Master Mix (2X): 5 x 1.0 mL

Use By: 6 months from date of receipt

DESCRIPTION

Tonbo's FastPROBE™ qPCR No-ROX Master Mix is a ready-to-use cocktail for the amplification and detection of DNA on real-time instruments that do not incorporate a ROX passive reference dye. 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. Tonbo's FastPROBE qPCR No-ROX Master Mix is ideal for both standard and fast-cycling real-time PCR applications using sequence-specific fluorogenic probes and reliably delivers excellent signal to noise ratios, rapid extension times for early C_t values, and enhanced sensitivity.

Tonbo's FastPROBE qPCR No-ROX is a robust, convenient high performance Master Mix optimized for real-time PCR that delivers enhanced fluorescence, speed, sensitivity and linearity. The FastPROBE qPCR No-ROX Master Mix can be used for the absolute quantitation of gene expression from a variety of templates and is ideal for low copy number detection, microarray validation, SNP genotyping and multiplexing.

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. The 2X Master Mix be stored at 4°C for up to 1 month.

BIOLOGICAL SOURCE

The Classic++ Hot Start *Taq* polymerase 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.*

INSTRUMENT COMPATIBILITY

Tonbo's FastPROBE qPCR No-ROX Master Mix can be used on a variety of real-time instruments that do not require a ROX reference dye. **Please Note**: FastPROBE qPCR No-ROX Master Mix is NOT compatible with instruments that require high (500 nM) or low (50 nM) concentrations of ROX dye. We offer our FastPROBE qPCR Hi-ROX Master Mix (Cat. No. 31-5112) or FastPROBE qPCR Lo-ROX Master Mix (Cat. No. 31-5111) for use with those instruments. Please see the Real-Time PCR Instrument Compatibility Guide (pg. 3) for additional information.

APPLICATION NOTES

Master Mix: FastPROBE qPCR No-ROX Master Mix (2X) contains contains Classic++ Hot Start *Taq* DNA Polymerase, Classic++ Hot Start mAbs, 6 mM $MgCl_2$ and 2 mM dNTPs 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.

ROX: No ROX dye is provided in this Master Mix. 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 guanosine residues and choose a probe close to the 5' primer. For each reaction, a final primer concentration of 0.2 - 0.6 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 less than 100 ng of cDNA, or less than 1 µg of genomic DNA.

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 reaction mix. If working with final reaction volumes greater than 20 µL, scale component volumes accordingly.
2. As applicable, transfer the recommended volume of master mix, primers and sample template DNA 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 in the channel appropriate to the probe reporter dye.

Table 1. Reaction Preparation

Reagent	20 µL reaction	Final Concentration	Notes
FastPROBE qPCR Master Mix (2X)	10 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	
Template DNA	<100 ng cDNA <1 µg genomic	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	95°C	2 minutes	Polymerase activation, 2 minutes for cDNA & 3 minutes for genomic
40	95°C	5 seconds	Denaturation
	60°C - 65°C	20-30 seconds	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 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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