

## TECHNICAL DATA SHEET

# CYBRFast™ qPCR Lo-ROX Master Mix

Catalog Numbers: 31-5101-0100R  
31-5101-0500R

## PRODUCT INFORMATION

**Contents:** 31-5101-0100R (100 rxns)  
CYBRFast™ qPCR Lo-ROX Master Mix (2X): 1 x 1.0 mL

31-5101-0500R (500 rxns)  
CYBRFast™ qPCR Lo-ROX Master Mix (2X): 5 x 1.0 mL

**Use By:** 6 months from date of receipt

## DESCRIPTION

Tonbo's CYBRFast™ qPCR Lo-ROX Master Mix is a ready-to-use cocktail for the amplification and detection of DNA on real-time instruments that support normalization with a standard ROX passive reference dye at a low final concentration of 50 nM. CYBRFast™ Hot Start *Taq* DNA Polymerase, a next generation thermostable DNA 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. 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 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 C<sub>t</sub> values and enhanced sensitivity. Convenient and reliable, Tonbo's CYBRFast qPCR Lo-ROX Master Mix is ideal for real-time PCR amplification and absolute quantification of DNA from a variety of templates, including those with GC and AT rich regions.

Tonbo's CYBRFast qPCR Lo-ROX is a robust, high performance Master Mix optimized for real-time PCR that delivers enhanced fluorescence, speed, sensitivity and linearity. The CYBRFast qPCR Lo-ROX Master Mix can be used for quantitation of gene expression and is ideal for low copy number detection, microarray validation, gene knock-down validation and quantification of Next Generation Sequencing libraries.

## STORAGE

Store reagent 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 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 CYBRFast Hot Start *Taq* DNA 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 aquaticus*. 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 aquaticus*.

## INSTRUMENT COMPATIBILITY

Tonbo's CYBRFast qPCR Lo-ROX Master Mix can be used on a wide variety of real-time instruments including those that do not have a ROX channel. **Please Note:** This Master Mix is NOT compatible with instruments that require a high concentration ROX dye. We offer our CYBRFast qPCR Hi-ROX Master Mix (Cat. No. 31-5102) for use with those instruments. Please see the Real-Time PCR Instrument Compatibility Guide (pg. 3) for additional information.

## APPLICATION NOTES

**Master Mix:** CYBRFast qPCR Lo-ROX Master Mix (2X) contains CYBRFast Hot Start *Taq* DNA Polymerase, CYBRFast Hot Start mAbs, CYBRFast GREEN fluorescent dye, 6 mM MgCl<sub>2</sub>, 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<sub>2</sub> or enhancers to the reaction mix.

**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 (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 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 Temperature:** 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 SYBR Green or FAM channel.

**Table 1. Reaction Preparation**

Reagent	20 µL reaction	Final Concentration	Notes
CYBRFast 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	
Template DNA	<100 ng cDNA <1 µg genomic	variable	See above for template considerations
Nuclease free dH <sub>2</sub> O	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
	95°C	5 seconds	Denaturation
40	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

**TECHNICAL SUPPORT**

Please provide the following information to [support@tonbobio.com](mailto: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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