2x qRT-PCR Master mix

Cat No	Description	qPCR Instruments
QRP001R 5 ml	2X qRT-PCR master mix with ROX	ABI,7000,7300,7700 7900,stepOne Plus StepOne™ Eppendorf Realplex 4
		ABI7500 Stratagene Mx3000, Mx3005, Mx4000
QRP001 5 ml	2x qRT-PCR master mix	BioRad CFX96 Roche LightCycler 480 MJ Research Opticon and Opticon 2 MJ Research Chromo 4 Corbett Rotor-gene 600,3000 Eppendorf Realplex 2Product Application

Product Description

2x qRT-PCR Master mix is designed for quantitative real-time analysis of DNA samples. • Strong signals and high sensitivity due to fluorescent dye.

- High specificity - no primer dimers, no NTC signal.
- Optimized 2x qRT-PCR Master mixes for different real-time PCR instruments.

Master mix formulations optimized for different machines.

2x qRT-PCR Master mix is ideally suited for:

- Gene expression analysis
- Microarray validation

Kit Components

2x qRT-PCR Master mix is a 2X mix of dNTPs, Hotstart Taq polymerase, M-MLV RTase, $MgCl_2$, fluorescent detection dye, reference dye (optional), and proprietary buffer components.

Storage:

long time:-20˚C
short time 4˚C

Recommended Protocol

Thaw 2x qRT-PCR Master mix, template DNA, RNase-free water and primer on ice. Mix each solution well. Prior to the experiment, It is prudent to carefully optimize experiment Conditions and to Include controls at every stage. See pre-protocol considerations for details. This standard protocol applies to a single reaction where only template, primers and water need to be added to the 2X qRT-PCR Master mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1.Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.

2. Assemble reaction tubes on ice.

3. Prepare a reaction Master mix using the following:

Components	Volume/Reaction	Final Concentration
2x qRT-PCR Master mix	10-25 μl	1X
Primer A	Variable	100-500nM
Primer B	Variable	100-500nM
Sterile water	Variable	
Template RNA	Variable	≤ 500ng/rx
Total Volume	20-50 μl	

A. Two-step fast cycling protocol

This cycling protocol should be applicable to most amplifications where the primer Tm's are designed to be 60 ºC. Melt curves may be performed by following instructions provided for your instrument.

Step	Temperature	Duration	Cycles
Rever Transcription	42˚C	5-15min	1
Enzyme activation	95˚C	10min	1
Denature	95˚C	5sec	40-45
Anneal/extension	60˚C	30sec	
Melting curve	According to the instrument guidelines		

B. Three-step fast cycling protocol

This cycling protocol can be used if you would like to have the extension step to be performed at a higher temperature than the annealing step. For example, if you have relatively long primers that tend to anneal non-specifically, carrying out the extension step at a higher temperature can reduce nonspecific amplification. Melt curves may be performed by following instructions provided for your instrument.

Step	Temperature	Duration	Cycles
Rever Transcription	42˚C	5-15min	1
Enzyme activation	95˚C	10min	1
Denature	95˚C	5sec	40-45
Anneal	60˚C	5sec	
extension	72˚C	25sec	
Melting curve	According to the instrument guidelines		

Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and to avoid repeated freeze-thaw cycles
- 2x qRT-PCR Master mix components are light sensitive; avoid exposure to light
- Start PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to PCR reactions

NOTE: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

NOTE: Shorter annealing step time (<10sec) can be used for amplicon <100bp.

For reseach use only

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