



## BioDiamond 2x qPCR Master mix

Cat No. DMDRT001  
Pack size: 4x 1.25 ml

qPCR Instruments:

BioRad CFX96, Roche LightCycler 480, MJ Research Opticon and Opticon 2, MJ Research Chromo 4, Corbett Rotor-gene 600,3000 and Eppendorf Realplex 2Product Application.

### Product Description

2x qPCR 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 qPCR Master mixes for different real-time PCR instruments. Master mix formulations optimized for different machines.

2x qPCR Master mix is ideally suited for:

- Gene expression analysis
- Microarray validation
- Viral load determination qRT-PCR

### Kit Components

2x qPCR Master mix is a 2X mix of dNTPs, Hotstart Taq polymerase, MgCl<sub>2</sub>, fluorescent detection dye, reference dye(optional), and proprietary buffer components.

### Storage

long time: -20°C  
short time: 4°C

### Recommended Protocol

Thaw 2x qPCR 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 qPCR 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 qPCR Master mix	10-25µl	1X
Primer A	Variable	100-500nM
Primer B	Variable	100-500nM
Sterile water	Variable	
Template DNA	Variable	≤ 500ng/rx
Total Volume	20-50µl	

### A. Two-step fast cycling protocol

This cycling protocol should be applicable to most amplifications where the primer T<sub>m</sub>'s are designed to be 60 °C. Melt curves may be performed by following instructions provided for your instrument.

Step	Temperature	Duration	Cycles
Enzyme activation	95°C	10min	1
Denature	95°C	5sec	40
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
Enzyme activation	95°C	10min	1
Denature	95°C	5sec	40
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 qPCR 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:

1. 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.
2. Shorter annealing step time (<10sec) can be used for amplicon <100bp.

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