

Store at  
-20°C



## Probe 2x qPCR Master mix

light

| Cat No                       | Description  | qPCR Instruments  |
|------------------------------|--|---|
| PRP0500R<br>5 ml<br>5X 5 ml  | Probe2X qPCR master mix with ROX                   | ABI,7000,7300,7700<br>7900,stepOne Plus<br>StepOne™<br>Eppendorf Realplex 4   |
| PRP0500LR<br>5 ml<br>5X 5 ml | Probe2x qPCR master mix with low concentration ROX | ABI7500<br>Stratagene Mx3000,<br>Mx3005, Mx4000   |
| PRP0500<br>5 ml<br>5X 5 ml   | Probe2x qPCR master mix                            | BioRad CFX96<br>Roche LightCycler 480<br>MJ Research Opticon<br>and Opticon 2<br>MJ Research Chromo 4<br>Corbett Rotor-gene<br>600,3000<br>Eppendorf Realplex<br>2Product Application |

### Product Description

Probe 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 Probe 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

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

### Storage:

long time:-20°C  
short time 4°C

### Recommended Protocol

Thaw Probe 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 Probe 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 |
|--------------------------|-----------------|---------------------|
| Probe 2x qPCR Master mix | 10-25 $\mu$ l   | 1X                  |
| Primer A                 | Variable        | 100-500nM           |
| Primer B                 | Variable        | 100-500nM           |
| probe                    | Variable        | 100-250nM           |
| Sterile water            | Variable        |                     |
| Template DNA             | Variable        | $\leq$ 500ng/rx     |
| Total Volume             | 20-50 $\mu$ l   |                     |

### 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
- Probe 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:** 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.

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