# 2X qPCR Master Mix – Hot Start

**Product #:** Bi2M-2xqPCR
**Quantity:** 1 mL

## Product Description

- The 2X qPCR Mix is a concentrated solution of *Taq* DNA polymerase, dNTPs, SYBR® Green and all other components required for Real-Time qPCR, except DNA template and primers.
- The SYBR® Green dye binds to double-stranded DNA formed during the PCR allowing for the real-time monitoring of reaction progress. The master mix also includes a non-interfering and visible tracking dye that helps to eliminate pipetting errors.
- The Taq DNA Polymerase is heat-activated and blocked by the aptamer method. This feature effectively inhibits nonspecific amplification under low temperature conditions.
- Combined with the optimized reaction buffer, the Taq DNA Polymerase ensures high specificity and sensitivity in qPCR reactions. The product enables the generation of accurate, reproducible, and reliable standard curves over a wide quantitative range for target gene analysis.
- The 2X qPCR Mix contains a special ROX Passive Reference Dye suitable for use in all qPCR instruments. There is no need to adjust the ROX concentration for different instruments.

## Storage

- Store at -20 $^{0}$C.

## Components Provided

| | |
|---|---|
| **2X qPCR Master Mix** | 1 mL |

## Important Preparatory Notes (PLEASE READ CAREFULLY)

1. It is recommended that all reaction components be assembled on ice.
2. Obtain DNA or cDNA of interest through a preferred DNA extraction and purification technique.
3. Prepare fresh dilutions of DNA or cDNA in either water or TE buffer.
4. We recommend running each DNA standard and sample in triplicate.
5. We recommend using filtered nuclease-free pipette tips.

## Protocol

1. Thaw **2X qPCR Master Mix**, primers, and DNA. Once thawed, gently mix each component by inversion, pipetting, or mild vortexing.
2. Calculate the total volume required for the desired number of reactions, adding a 10% surplus, and prepare the assay mix of all components except DNA template accordingly. Thoroughly but gently mix by pipetting. Ensure liquid is collected at the bottom of the tube by brief centrifugation.

| COMPONENT | 20µL rxn | 50µL rxn | FINAL CONCENTRATION |
|---|---|---|---|
| **2X qPCR Master Mix** | 10µL | 25µL | 1X |
| **Forward Primer (10µM)** | 0.4µL | 1µL | 0.2 µM |
| **Reverse Primer (10µM)** | 0.4µL | 1µL | 0.2 µM |
| **Nuclease-Free Water** | Add to 15 µL | Add to 45 µL | |

3. Dispense assay mix into qPCR tubes (Cat# PCR-02-FC), strip-tubes (Cat# PTS-200) or plates (Cat# WP-96-PCR-200-HS or WP-96-PCR-100-NS). For optimal results, maintain precise and consistent pipetting volumes, and minimize the formation of bubbles.
4. Add 5 µL DNA template to the qPCR tubes or plates.

| COMPONENT | 20µL rxn | 50µL rxn | FINAL CONCENTRATION |
|---|---|---|---|
| **Template** | Variable | Variable | Typically 1 to 10 ng<br>(do not exceed 100 ng) |
| **Nuclease-Free Water** | Add to 5 µL | Add to 5 µL | |

5. Seal tubes with flat, optically transparent caps, and seal plates with optically transparent film (Cat# PPH). Take care to seal plate edges and corners properly to prevent evaporation-related artifacts.
6. Briefly spin tubes or plates to eliminate bubbles and gather liquid (1 minute at 1,000 RCF).

7. Program the real-time instrument with the specified thermocycling protocol (refer to the tables below).
   I. If amplification specificity needs to be improved, **two-step** procedure or annealing temperature can be used. Two-step procedure is recommended.
   II. To improve the amplification efficiency, a **three-step** procedure or extension time can be used.
   III. Ensure a plate read is scheduled at the conclusion of the extension step.

| TWO STEP METHOD (RECOMMENDED) | | | | |
|---|---|---|---|---|
| **Stage** | **Step** | **Cycle #** | **Temperature** | **Time** |
| **Stage 1** | Initial Denaturation | 1 | 95℃ | 30 sec |
| **Stage 2** | Denaturation | 40 | 95℃ | 15 sec |
| | Annealing-Extension | | 60℃ | 30 sec **(+ Plate Read)** |
| **Stage 3** | Melt Curve | 1 | Instrument default Settings | |

| THREE STEP METHOD | | | | |
|---|---|---|---|---|
| **Stage** | **Step** | **Cycle #** | **Temperature** | **Time** |
| **Stage 1** | Initial Denaturation | 1 | 95℃ | 30 sec |
| **Stage 2** | Denaturation | 40 | 95℃ | 15 sec |
| | Annealing | | 55-65℃ | 10 sec |
| | Extension | | 72℃ | 30 sec **(+ Plate Read)** |
| **Stage 3** | Melt Curve | 1 | Instrument default Settings | |

## Optimization of PCR Conditions

1. **Primers:** Typically, a satisfactory amplification outcome is achieved with a final concentration of 0.2µM. In instances of suboptimal reaction performance, primer concentration can be fine-tuned within the range of 0.2-1.0µM. Excessive primer concentration increases the probability of mispriming and the generation of non-specific PCR products.
2. **DNA Template:** The quantity of template added depends on the copy number of the target gene in the template solution, and the suitable amount of template addition is determined through

gradient dilution. In a 20µL reaction system, the optimal amount of template DNA was found to be less than 10 ng (0.01-1 ng for both plasmid and viral DNA, and 0.1-100 ng for genomic DNA). When using cDNA from an RT-PCR reaction as a template, the addition amount should not exceed 10% of the total volume of the PCR reaction solution.

3. **Initial DNA Denaturation:** Template DNA needs to be completely denatured at the beginning of the PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95 $^0$C is sufficient.
4. **Denaturation:** A DNA denaturation time of 30 seconds per cycle at 95 $^0$C is normally sufficient. DNA template, which are GC-rich may need prolonged denaturation times.
5. **Primer Annealing:** The annealing temperature should be 5 $^0$C lower than the melting temperature (Tm) of the primers. Annealing times of 30 seconds are normally sufficient. If nonspecific PCR products appear, the annealing temperature should be optimized via gradient or stepwise 1-2 $^0$C increments.
6. **Extension:** The optimal extension temperature is 72 $^0$C. The recommended extension time is 1 min/kb of expected product.
7. **Number of Cycles:** Typically, 40 cycles are sufficient to obtain adequate amounts of product. In cases where template concentration is low, an increased number of cycles may be required.

## PCR Machine Compatibility

1. ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900 HT Fast, StepOne™, StepOne Plus™, 7500/7500 Fast, ViiA 7™, QuantStudio™ series, PikoRealTM Cycler;
2. Stratagene: Mx3000P®, 3005P™, 4000™;
3. Bio-Rad: CFX96™, CFX384™, iCycler iQ™, iQ5™, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™;
4. Eppendorf: Realplex 2s, Mastercycler® ep, realplex;
5. IIIumina: Eco QPCR;
6. Cepheid: SmartCycler®;
7. Qiagen Corbett: Rotor-Gene® series;
8. Roche: LightCycler™ series;
9. Takara: Thermal Cycler Dice series;
10. Analytikjena: qTOWER series;
11. qTOWER: LineGene series.