# BlazeTaq™ Probe qPCR Master Mix

Easy-to-use mixes for probe-based real-time PCR

| With ROX Reference Dye | Without ROX Reference Dye |
|---|---|
| Cat.No.**QP035** (20 µl × 100 reactions) | Cat.No.**QP045** (20 µl × 100 reactions) |
| Cat.No.**QP036** (20 µl × 200 reactions) | Cat.No.**QP046** (20 µl × 200 reactions) |
| Cat.No.**QP037** (20 µl × 600 reactions) | Cat.No.**QP047** (20 µl × 600 reactions) |
| Cat.No.**QP039** (20 µl × 1000 reactions) | Cat.No.**QP049** (20 µl × 1000 reactions) |

Performance optimized for All-In-One™ qPCR Primers, ExProfile™ Gene qPCR Arrays.

## User Manual

GeneCopoeia, Inc.
9620 Medical Center Drive, #101
Rockville, MD 20850
USA

301-762-0888
866-360-9531

inquiry@genecopoeia.com

www.genecopoeia.com

# BlazeTaq™ Probe qPCR Master Mix



## I. Description

BlazeTaq™Probe qPCR Master Mix is a probe-based real-time PCR assay. This product is a ready-to-use 5× premix solution that allows for rapid, specific quantitation of template DNA by simply adding your DNA template, primers, and sterile water. The product incorporates a new antibody-modified BlazeTaq™ hot-start DNA polymerase that can block enzyme activity at room temperature and block nonspecific amplification more efficiently. With initial denaturation at 95 ºC for 2 min, polymerase can be fully activated. In addition, the optimized buffer system significantly improves the sensitivity and repeatability of the Real Time PCR reactions, and has the characteristics of high amplification efficiency, strong specificity and wide linear dynamic range. Also it can effectively inhibit the production of primer dimers and further enhance reliability of experimental results.

## II. Related Products

GeneCopoeia offers comprehensive solutions for studying gene expression. A careful process of co- development ensures that they work well together and provide robust and reproducible results.

| Product | Description |
|---|---|
| BlazeTaq™ SYBR® Green qPCR mix 2.0 | SYBR Green-based real-time quantitative PCR Mix |
| SureScript™ First-Strand cDNA Synthesis Kit | Reverse transcribe mRNA into first–stand cDNA |
| All-in-One™ qPCR Primers | Validated, gene-specific primers ensure specificity and sensitivity (human, mouse and rat) |
| ExProfile™ Gene qPCR Arrays | High-throughput or focused group profiling of gene expression |
| All-in-One™ miRNA First-Strand cDNA Synthesis Kit | Reverse transcribe miRNA into first –stand cDNA |
| All-in-One™ miRNA qRT-PCR Detection Kits | SYBR Green-based detection kit accurately quantifies miRNA expression |
| All-in-One™ miRNA qPCR Primers | Validated human, mouse, rat miRNA primers for robust, reproducible and reliable quantitation of miRNA activity |
| miProfile™ miRNA qPCR Arrays | High-throughput or focused group profiling of miRNA expression |
| RNAzol® RT RNA Isolation Reagent | Easy isolation of mRNA, microRNA or total RNA |

## III. Contents and Storage

Contents and storage recommendations for the BlazeTaq™ Probe qPCR Master Mix are provided in the following table.

For kits with the catalog number **QP035**

| Catalog Number | Contents | Quantity | Storage temperature/ conditions |
|---|---|---|---|
| QP035-01 | BlazeTaq™ Probe PCR Mix (5×) | 400 μL | Store at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at - 80°C in aliquots. Avoid repeated freezing/thawing. |
| QP035-02 | ROX Reference Dye (30 μM) | 40 μL | Store in dark at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing. |

For kits with the catalog number **QP036**, **QP037** and **QP039**

| Catalog Number | Contents | Quantity | Storage temperature/ conditions |
|---|---|---|---|
| QP036-01 | BlazeTaq™ ProbePCR Mix (5×) | 1×800 μL<br>3×(1×800 μL)<br>5×(1×800 μL) | Store at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing. |
| QP001-02 | ROX Reference Dye (30 μM) | 1×80 μL<br>3×(1×80 μL)<br>5×(1×80 μL) | Store in dark at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing. |

Contents and storage recommendations for the BlazeTaq™ Probe qPCR Master Mix (without ROX) are provided in the following table.

For kits with the catalog number **QP045**

| Catalog Number | Contents | Quantity | Storage temperature/ conditions |
|---|---|---|---|
| QP045-01 | BlazeTaq™ Probe PCR Mix (5×) | 400 μL | Store at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing. |

For kits with the catalog number **QP046**, **QP047** and **QP049**

| Catalog Number | Contents | Quantity | Storage temperature/ conditions |
|---|---|---|---|
| QP046-01 | BlazeTaq™ Probe PCR Mix (5×) | 1×800 μL<br>3×(1×800 μL)<br>5×(1×800 μL) | Store at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing. |

### Required Materials (Not Included)

- Template
- Target-specific forward and reverse primers, qPCR probes
- $ddH_2O$ (Nuclease-free)
- PCR strip tubes or microcentrifuge tubes (for reaction setup)
- qPCR tubes or plates (Rnase-free)
- nuclease-free pipettes
- Microcentrifuge
- qPCR instrument

## IV. Preparation

**Wearing a lab coat, disposable gloves and protective goggles are recommended when handling chemicals.**

### IMPORTANT NOTES:

1. When using the BlazeTaq™ Probe qPCR Master Mix with miProfile miRNA qPCR Arrays and All-in-One miRNA First- Strand cDNA Synthesis Kit for miRNA expression profiling, please follow the miProfile miRNA qPCR array user manual for the complete instruction.
2. Store the kit at –20°C. Avoid storage or leaving reagents at 4°C or room temperature. Avoid light exposure at all times.
3. Mix reagents thoroughly by gently inverting tubes several times while avoiding bubbles, and then briefly centrifuge before use.
4. Prepare the reaction mix with PCR grade water.
5. Follow standard procedures for PCR to avoid nucleic acid contamination and non-specific amplification.
6. Read all procedures before setting up the PCR reaction.

## V. Procedure

**1.** Thaw the BlazeTaq™ Probe qPCR Master Mix (5× ) and other reaction components at room temperature, then place on ice. After thawing completely, briefly mix each component by inversion, pipetting or gentle vortexing.

**2.** Prepare the qPCR reaction mix according to the table below. Add the following reagents into an RNase-free reaction tube or plate which has been pre-cooled on ice.

| Reagent | Volume | Final concentration |
|---|---|---|
| BlazeTaq™ Probe qPCR Mix [a] | 4 µl | 1× |
| PCR forward primer (2 µM) [b] | 2 µl | 0.2 µM [c] |
| PCR reverse primer (2 µM) | 2 µl | 0.2 µM |
| Template | 2 µL | variable |
| Probe(10 µM)[d] | 0.2 µL | 0.1 µM |
| ROX Reference Dye [e](30 µM), *optional* | 0.4 - 0.1 µL | 600 nM - 150 nM |
| Water (double distilled) | | |
| ▪ Not using ROX Reference Dye | 9.8 µL | |
| ▪ Using ROX Reference Dye | 9.4-9.7 µL | |
| **Total** | **20 µL** | |

a. The kit has been optimized for a final reaction volume of 20 µl. If the total reaction volume is changed, maintain each component in the proper proportion.

b. Primers are important considerations to ensure success with real-time PCR. All-in-One™ human, mouse and rat primer sets from GeneCopoeia have been validated to provide specific and sensitive amplification even with low copy number genes. For designing your own primers, you may wish to use Oligo primer analysis software (Molecular Biology Insights) or Primer Premier software (Premier Biosoft International).

c. Primer concentration should be in the range of 0.2 to 0.6 µM. In general, a PCR reaction using 0.2 µM primers produces good results. If the PCR efficiency is low, consider increasing primer concentration. However, keep in mind that non-specific PCR products may also increase with increased primer concentration.

d. The concentration of probe is related to the type of Real Time PCR amplifier, probe and fluorescent labeling substance used. Please refer to the Instrument Instructions or the specific requirements of each fluorescent probe for practical use. Generally adjust between 50-250 nM.

e. ROX Reference Dye is only supplied in BlazeTaq™ Probe qPCR Master Mix (Cat. Nos. QP036, QP037 and QP039). It should be added only for qPCR instruments that require ROX for calibration.

ROX Reference Dye provides an internal reference to which the reporter-dye signal can be normalized during data analysis. Normalization is necessary to correct for fluorescence fluctuations due to changes in concentration or volume. Adjust the ROX Reference Dye to optimal concentration according to different qPCR instruments.

| Instrument | ROX per 20 µl PCR Reaction | Final Concentration |
|---|---|---|
| BioRad iCycler, MyiQ, iQ5, CFX-96, CFX-384, Eppendorf Mastercycler realplex, Roche LightCycler 480, LightCycler 2.0 | None | No ROX |
| ABI PRISM 7000/7300/7700/7900HT and 7900HTFast, ABI Step One, ABI Step One Plus | 0.4 µl (0.2-0.4 µl) | 600 nM (300-600 nM) |
| ABI 7500, 7500 Fast, ABI ViiA7, Stratagene Mx3000P, Mx3005P, Mx4000 | 0.1 µl (0.02-0.1 µl) | 150 nM (30-150 nM) |

*For other instruments that need calibration with ROX but have not been listed out in the table, please optimize the concentration of ROX according to the guidelines of the specific instrument.*

**3.** Mix the qPCR reaction mix sufficiently and add to the PCR reaction tubes.

**4.** Briefly centrifuge to remove bubbles and make sure all the reagents are at the bottom of the reaction tubes/plates.

**5.** The following two-step method for programming the PCR reaction is recommended:

| Cycles | Steps | Temperature | Time | Detection |
|---|---|---|---|---|
| 1 | Initial Denaturation | 95°C | 2 min | No |
| 40 | Denaturation | 95°C | 10 sec | No |
| | Extension | 60°C | 30 sec | Yes |

Notes

i. The DNA polymerase used in the 5×BlazeTaq qPCR Mix is a special antibody modified hot-start enzyme. Incubation for 2 min at 95 °C will sufficiently activate the enzyme.

ii. Usually, 60°C recommended by default is used as the extension temperature in the reaction system. If the extension temperature is to be adjusted, it can be adjusted in the range of 60 - 65 °C according to the Tm value of the primer.

iii. The optimal fragment length to use for amplification during real-time PCR is in the range of 80- 150 bp. However, fragment lengths up to 500 bp are possible.

iv. The main condition for the above reaction are referred to the Roche LightCycler 480 qPCR instrument manual from Bio-Rad. If a qPCR instrument from another commercial source is used, please reference the instrument manual and adjust the extension time and melting curve conditions accordingly.

## VI. Example

**Objective**: The amplification efficiency and detection sensitivity of the BlazeTaq™ Probe qPCR Master Mix are assessed by examining the amplification result of ACTB, GAPDH and B2M from serially diluted cDNA from total RNA sample extracted from HeLa cells.

**Equipment**: Roche LightCycler 480-2 Real Time qPCR

**Procedure**:

1. The DNA standard was serially diluted to 6 concentrations ranging from 100 ng to 1 pg.
2. qPCR reaction mix was prepared as below.

| Reagent components | Volume |
|---|---|
| BlazeTaq™ Probe qPCR Master Mix (5×) | 4 µl |
| PCR forward primer (10 µM) | 0.5 µl |
| PCR reverse primer (10 µM) | 0.5 µl |
| Probe (6 µM) | 0.5 µl |
| $ddH_2O$ | 9.5 µl |
| **Total** | **15 µl** |

3. Mix the above reagents sufficiently. Aliquot to PCR tubes after a brief centrifugation.
4. Add 5 µl of the cDNA to each PCR tube. Use 5 µl $ddH_2O$ as a no-template control (NTC).
5. Program the real-time PCR reaction and corresponding reading conditions of the melting curve:

| Cycles | Steps | Temperature | Time | Detection |
|---|---|---|---|---|
| 1 | Initial Denaturation | 95°C | 20 min | No |
| 40 | Denaturation | 95°C | 10 sec | No |
| | Extension | 60°C | 30 sec | Yes |

6. Analyze the amplification curves after the qPCR experiment:

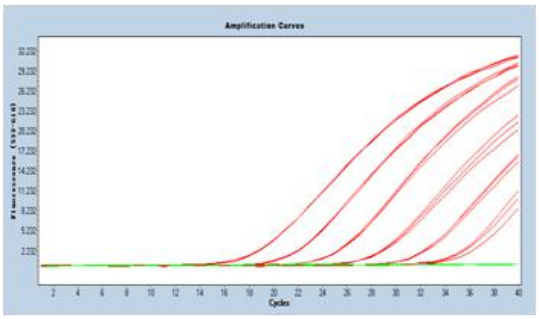


Figure 1. Amplification curves of ACTB

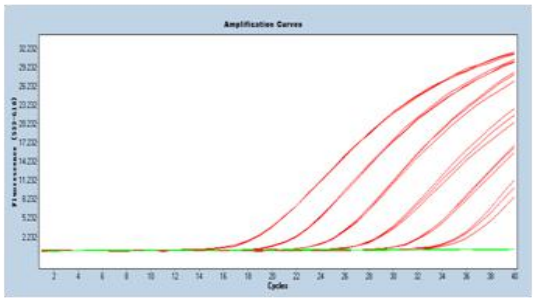


Figure 2. Amplification curves of GAPDH

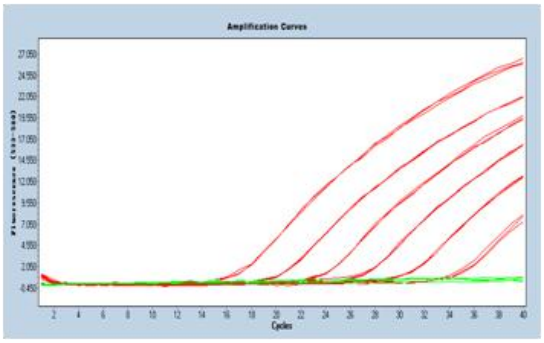


Figure 3. Amplification curves of B2M

7. Construct a standard curve using the Ct values from each amplification curve:

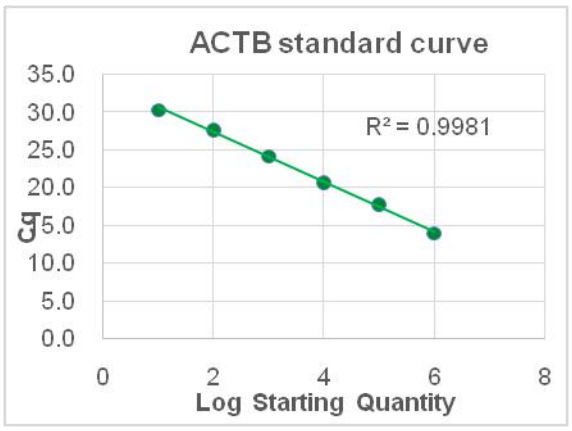


Figure 4. Standard curves of ACTB amplification

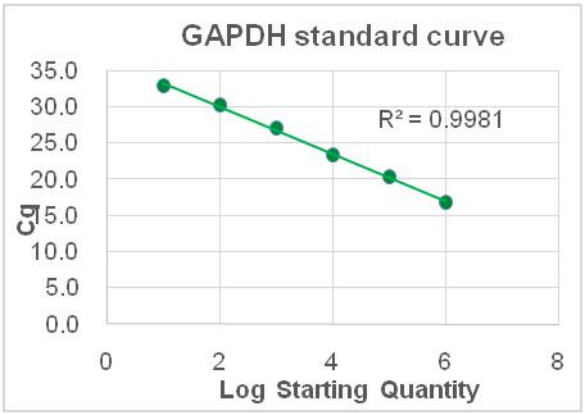


Figure 5. Standard curves of ACTB amplification

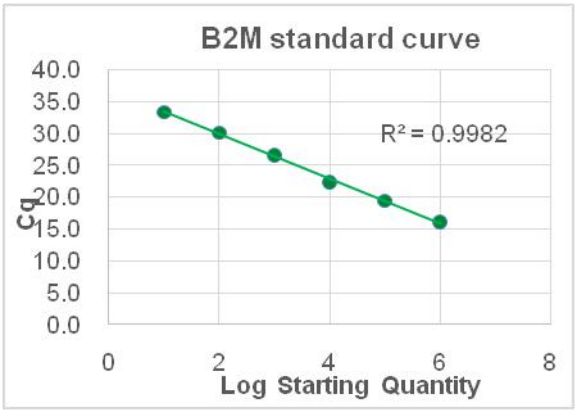


Figure 6. Standard curves of ACTB amplification

8. **Conclusion**: The peak values from the amplification show that BlazeTaq™ Probe qPCR Master Mix can detect ACTB, GAPDH and B2M from 1 pg total cDNA when using DNA as a template and that there is only a single amplified product, showing that very high sensitivity can be attained using the BlazeTaq Probe qPCR Master Mix. At the same time, high amplification efficiency is also shown by the good linear relationship among each concentration of serially diluted DNA standard..

## VII. Trouble Shooting Guide

<table>
<tr><td>Poor precision or failed qPCR reactions</td><td>
<ul>
<li>Make sure the initial denature time was set as 10min, sufficiently activating of the hot-start polymerase could avoid non-specific amplification and production of primer-dimers.</li>
<li>The fluorescence detection temperature may not be appropriate. Adjust accordingly.</li>
<li>The set up position for reaction samples in the real-time PCR instrument may not be right. Adjust accordingly.</li>
<li>PCR cycle conditions, primer concentration and primer sequences may not be appropriate. Adjust the primer concentration and annealing temperature. If this does not work, redesign the primers.</li>
<li>The template sample purity may not be adequate. Purify the template sample by phenol/chloroform extraction and ethanol precipitation. If the samples are reverse transcribed cDNA, set up the qPCR reaction with a diluted sample as other concentrated reagents in the RT reaction mixture may be interfering with the qPCR.</li>
<li>Try to use 3.0% agarose gel electrophoresis to check the qPCR products. Check the purity of the primers by electrophoresis or use PAGE-purified primers if the bands are diffused. One may also use phenol/chloroform extraction and ethanol precipitation methods to treat the primers before the experiment.</li>
</ul>
</td></tr>
<tr><td rowspan="2">Abnormal melting curves</td><td>
Signal in the blank (No Template Control) sample
<ul>
<li>There may be contamination of the positive samples in the qPCR reaction system if the $T_m$ of the melting curve of the blank control is the same as the positive control. Eliminate sample application error first. If the situation still persists, replace the PCR grade water and/or primers and/or use a new 5×BlazeTaq™ qPCR Mix.</li>
<li>If the $T_m$ of the melting curve of the blank control is lower than the positive control, the qPCR reaction may have produced nonspecific amplification such as primer-dimers. Prepare the qPCR reaction mix on ice and increase the temperature of fluorescence detection. If this does not work, redesign the primers.</li>
</ul>
</td></tr>
<tr><td>
Double peaks and multiple peaks in the melting curve of the positive control
<br>In the absence of other primers present in the reaction, double or multiple peaks in the melting curve of the positive control indicate that the qPCR reaction produced nonspecific amplification
</td></tr>
</table>

| | |
|---|---|
| | Prepare the qPCR reaction mix on ice; optimize the qPCR reaction conditions, for example, by increasing the annealing temperature, decreasing the primer concentration or increasing the fluorescence detection temperature (not more than the $T_m$ value of the expected product). If this does not work, redesign the forward primer.<br><br>**No peaks or abnormal peaks in the melting curve (or the amplification curves) of the positive control**<br><br>• Adjust the ROX Dye to optimized concentration according to instrument. |
| **No signal (Ct) or late appearing signal** | ● Not enough PCR cycles. For good sensitivity, one should generally set up more than 35 PCR cycles, but more than 45 cycles may result in too much background signal.<br>● The amount of template used may not be enough or the template may be degraded. Use the highest concentration possible of diluted template samples to set up the qPCR. At the same time, avoid freezing and thawing the samples repeatedly.<br>● The amplification efficiency is low and the qPCR reaction conditions are not optimal. Redesign the primers and optimize the reaction conditions. |

## VIII. Limited Use License and Warranty

**Limited Use License**

The following terms and conditions apply to use of all BlazeTaq™ Probe qPCR Master Mix (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

**Limited Warranty**

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.



GeneCopoeia, Inc.

9620 Medical Center Drive, #101

Rockville, MD 20850

Tel: 301-762-0888 Fax: 301-762-3888

Email: inquiry@genecopoeia.com

Web: www.genecopoeia.com