# BlasTaq™ Probe One-Step RT-qPCR

## Cat. No. G596

Store at -20°C.

## Product Description

**BlasTaq™ Probe One-Step RT-qPCR** provides convenience using the RT-qPCR Enzyme Mix and BlasTaq™ Probe 2X qPCR MasterMix for **both highly sensitive and specific reverse transcription, and TaqMan probe-based real-time qPCR amplification in a single reaction tube.** Our proprietary RT-qPCR Enzyme Mix contains stabilizers and enhancers that optimize the two reactions in a "single step". This offers the end user flexibility with RNA templates and primer selection, an efficient and easy-to-use set up, and a reliable alternative to conventional "two-step" sequential RT-qPCR. The MasterMix is suitable for SNP genotyping assays, gene expression analysis, microarray validation, and high throughput screening applications. ROX reference dye is provided separately, making it universally compatible with most qPCR instruments.

| Product Component | Quantity | Part No. |
|---|---|---|
| BlasTaq™ Probe 2X qPCR MasterMix | 1.25 ml | G890-1 |
| RT-qPCR Enzyme Mix | 100 rxn (40 µl) | P113 |
| ROX Reference Dye | 15 µl | P101 |
| Nuclease-Free $H_2O$ | 1.0 ml | P100 |

## Protocol

Reactions should be assembled in an RNase-free environment. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

The recommended amount of ROX Reference Dye to be added into the MasterMix may vary depending on the qPCR machine type:

- No ROX equipment: Not needed.
- Low ROX equipment: 1 µl/1.25 ml MasterMix.
- High ROX equipment: 11 µl/1.25 ml MasterMix.



1. Thoroughly thaw and mix individual components before use, and assemble reaction on ice.

| Component | Volume |
|---|---|
| BlasTaq™ Probe 2X qPCR MM | 10 µl |
| RT-qPCR Enzyme Mix | 0.4 µl |
| Forward Primer | Variable (100 – 500 nM) |
| Reverse Primer | Variable (100 – 500 nM) |
| TaqMan Probe | Variable (100 – 300 nM) |
| Total RNA or poly(A) + mRNA [1] | Variable (1 pg - 2 µg/rxn) |
| Nuclease-free $H_2O$ | up to 20 µl |

[1] Amplicon should be <150 bp in size.

2. Gently mix the reaction components and briefly centrifuge.
3. Thermocycling conditions for standard qPCR:

| Step | Temperature | Duration | | Cycle(s) |
|---|---|---|---|---|
| | | Standard | Fast | |
| cDNA Synthesis | 50°C | 15 min | 15 min | 1 |
| Enzyme Activation | 95°C | 3 min | 20 sec | 1 |
| Denaturation | 95°C | 15 sec | 1 sec | 40 |
| Annealing/Extension | 60°C | 1 min | 10 sec | |

## General Notes

- Both poly(A) + mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.
- For longer transcripts >9 kb, yields can be increased by incubating at 50°C for 30-50 minutes.
- RNA samples must be free of genomic DNA contamination.
- To remove RNA complementary to the cDNA, add 1 µl of *E. coli* RNase H (Cat. No. **E018**) and incubate at 37°C for 20 minutes.
- Use either the Standard or Fast qPCR program for your appropriate application.

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