



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 separate from the MasterMix, making this kit 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 ₂ O	1.0 ml	P100

Protocol

ROX Reference Dye is provided separate from the MasterMix, making this kit universally compatible with most qPCR instruments.

See **Rox Machine Compatibility** on our product page under the Documents tab on our website.

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.

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

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 – 500 nM)
Total RNA or poly(A) + mRNA ¹	Variable (1 pg - 2 µg/rxn)
Nuclease-Free H ₂ O	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 lengthening the cDNA synthesis incubation at 50°C to 30-60 minutes.
- RNA samples must be free of genomic DNA contamination.
- Use either the Standard or Fast qPCR program for your appropriate application.