GoTaq® Probe 2-Step RT-qPCR System

Instructions for Use of Product A6110.

Promega

Quick Protocol

Preparation of cDNA

Denaturation of RNA and Primers

1. On ice combine RNA template and primers.

Component	Volume
RNA template (DNA-free; up to 1µg/reaction)	1–5µl
Oligo(dT)$_{15}$ Primer (0.5µg/µl)	1µl
Random Primers (0.5µg/µl)	1µl
Nuclease-Free Water	to a final volume of 7µl

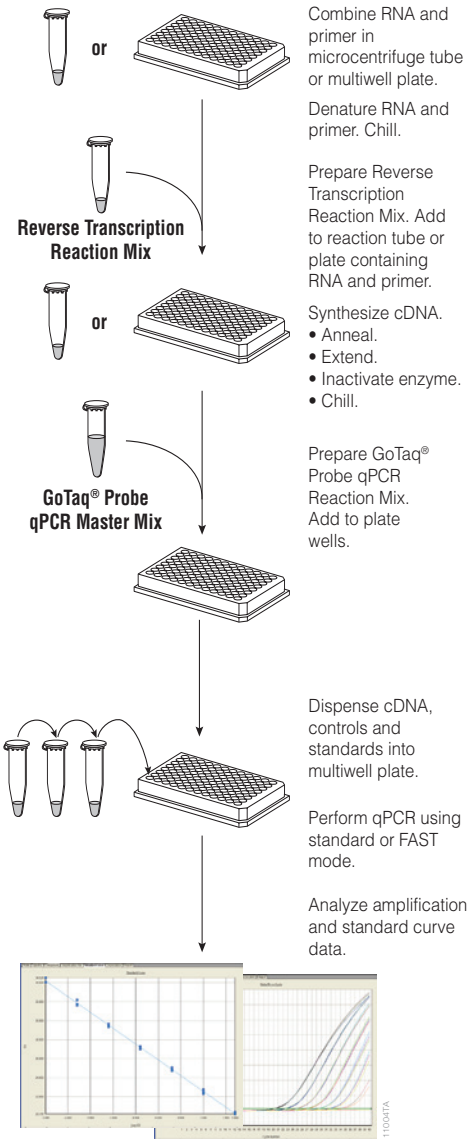
2. Denature the RNA and primers in a heating block at 70°C for 5 minutes. Immediately cool on ice for 5 minutes. Centrifuge for 10 seconds.
3. Store RNA and primers on ice prior to adding the Reverse Transcription Reaction Mix.

Primer Annealing and cDNA synthesis

4. Combine the following GoScript™ Reverse Transcription System components on ice, in the order shown. Prepare 13µl for each cDNA synthesis reaction to be performed. Vortex gently to mix and store on ice.

Component	Reverse Transcription Reaction Mix (Volume per Reaction)	Minus-Reverse Transcriptase Reaction Mix (Volume per Reaction)
Nuclease-Free Water	4.9µl	5.9µl
GoScript™ 5X Reaction Buffer	4µl	4µl
$MgCl_2$	1.6µl	1.6µl
PCR Nucleotide Mix	1µl	1µl
Recombinant RNasin® Ribonuclease Inhibitor	0.5µl	0.5µl
GoScript™ Reverse Transcriptase	1µl	1µl
Final volume	13µl	13µl

5. Add 13µl of the Reverse Transcription Reaction Mix to each RNA + primer tube for a final reaction volume of 20µl.
6. **Anneal:** Place tubes in a heating block at 25°C. Incubate for 5 minutes.
7. **Extend:** Incubate tubes in a controlled-temperature heating block at 42°C for 45 minutes.
8. **Inactivate Reverse Transcriptase:** Incubate tubes in heating block at 70°C for 15 minutes.
9. Proceed to qPCR amplification, or store cDNA at –20°C.



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GoTaq® Probe qPCR Protocol

Adding CXR Reference Dye to the GoTaq® Probe qPCR Master Mix (Optional)

If you wish to add CXR Reference Dye to your amplification reactions, we recommend adding an aliquot of concentrated CXR Reference Dye to the 1ml tube of GoTaq® Probe qPCR Master Mix at either a "low dye" or "high dye" concentration. Refer to the *GoTaq® Probe 2-Step RT-qPCR System Technical Manual* #TM380, Section 5.A, for detailed information.

Preparation of GoTaq® Probe qPCR Reaction Mix

GoTaq® Probe qPCR Master Mix uses a hot-start chemistry, allowing reaction setup to be done at room temperature.

1. Thaw GoTaq® Probe qPCR Master Mix and Nuclease-Free Water. **Do not** thaw the GoTaq® Probe qPCR Master Mix at temperatures above room temperature.
2. Vortex GoTaq® Probe qPCR Master Mix for 3–5 seconds.
3. Determine the number of reactions to prepare, including negative controls, and then increase the number by 1–2 reactions to compensate for pipetting error.
4. Prepare the reaction (minus cDNA template) by combining GoTaq® Probe qPCR Master Mix, PCR primers, hydrolysis probe and Nuclease-Free Water as described below. The DNA template is added in Step 6. Vortex briefly to mix.

Component	Volume	Final Concentration
GoTaq® Probe qPCR Master Mix, 2X	10µl	1X
Forward primer (20X)	1µl	200nM–1µM
Reverse primer (20X)	1µl	200nM–1µM
Hydrolysis probe (20X)	1µl	100–300nM
Template cDNA	2–5µl	≤250ng
Nuclease-Free Water	to a final volume of 20µl	

5. Add reaction mix (minus cDNA template) to each PCR tube or well of an optical-grade PCR plate.
6. Add cDNA template to the appropriate wells of the reaction plate. Add water to the no-template control reactions.
7. Seal the tubes or plates and centrifuge briefly to collect components to the bottom of the tubes or wells. Protect from light or elevated temperatures. Samples are now ready for thermal cycling.

Standard Thermal Cycling Guidelines

Step	Cycles	Temperature	Time
GoTaq® DNA Polymerase activation	1	95°C	2 minutes
Denaturation	40	95°C	15 seconds
Annealing/Extension		60°C	1 minute

For FAST cycling conditions, refer to the *GoTaq® Probe 2-Step RT-qPCR System Technical Manual* #TM380, Section 6.

Additional protocol information is in Technical Manual #TM380, available online at: **www.promega.com**

