GoTaq® Probe 1-Step RT-qPCR Protocol

Addition of CXR Reference Dye to GoTaq® 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 (Cat.# A6120) or 12.5ml bottle (Cat.# A6121) of GoTaq® Probe qPCR Master Mix at either a "low dye" or "high dye" concentration. Refer to the *GoTaq® Probe 1-Step RT-qPCR System Technical Manual* #TM379, Section 4.A, for detailed information.

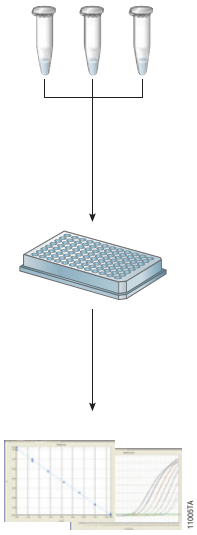
Preparation of GoTaq® Probe 1-Step RT-qPCR Reaction Mix

The 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 Master Mix above room temperature.
2. Vortex the GoTaq® Probe qPCR Master Mix for 3–5 seconds.
3. Determine the number of reactions to prepare, including negative controls, then increase the number by 1–2 reactions to compensate for pipetting error.

Component	Volume	Final Concentration
GoTaq® Probe qPCR Master Mix, 2X	10µl	1X
GoScript™ RT Mix for 1-Step RT-qPCR (50X)	0.4µl	1X
Forward primer (20X)	1µl	200nM–1µM
Reverse primer (20X)	1µl	200nM–1µM
Hydrolysis probe (20X)	1µl	100nM–300nM
RNA Template	2–5µl	10pg–1µg
Nuclease-Free Water	to a final volume of 20µl	

4. Prepare the reaction (minus RNA template) by combining GoTaq® Probe qPCR Master Mix, PCR primers, hydrolysis probe, and Nuclease-Free Water. Vortex briefly to mix.
5. Add reaction mix (minus RNA template) to each PCR tube or well of an optical grade PCR plate.
6. Add RNA template to the sample 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 before thermal cycling.



GoTaq® Probe 1-Step RT-qPCR Protocol (continued)

Thermal Cycling

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results.

Standard Cycling Conditions

Step	Cycles	Temperature	Time
Reverse Transcription	1	45°C	15 minutes
RT inactivation/GoTaq® Polymerase activation	1	95°C	2 minutes
Denaturation	40	95°C	15 seconds
Annealing/Extension		60°C	1 minute

FAST Cycling Conditions

Step	Cycles	Temperature	Time
Reverse Transcription	1	45°C	5 minutes
RT inactivation/GoTaq® Polymerase activation	1	95°C	2 minutes
Denaturation	40	95°C	3 seconds
Annealing/Extension		60°C	30 seconds

Additional protocol information in Technical Manual #TM379, available online at: **www.promega.com**

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