GoTaq® 1-Step RT-qPCR System

INSTRUCTIONS FOR USE OF PRODUCT A6020.

Quick PROTOCOL

For more information, see the *GoTaq® 1-Step RT-qPCR System Technical Manual* #TM355, available at: **www.promega.com/resources/protocols/**

Protocol

Figure 1. Overview of the GoTaq® 1-Step RT-qPCR Protocol.

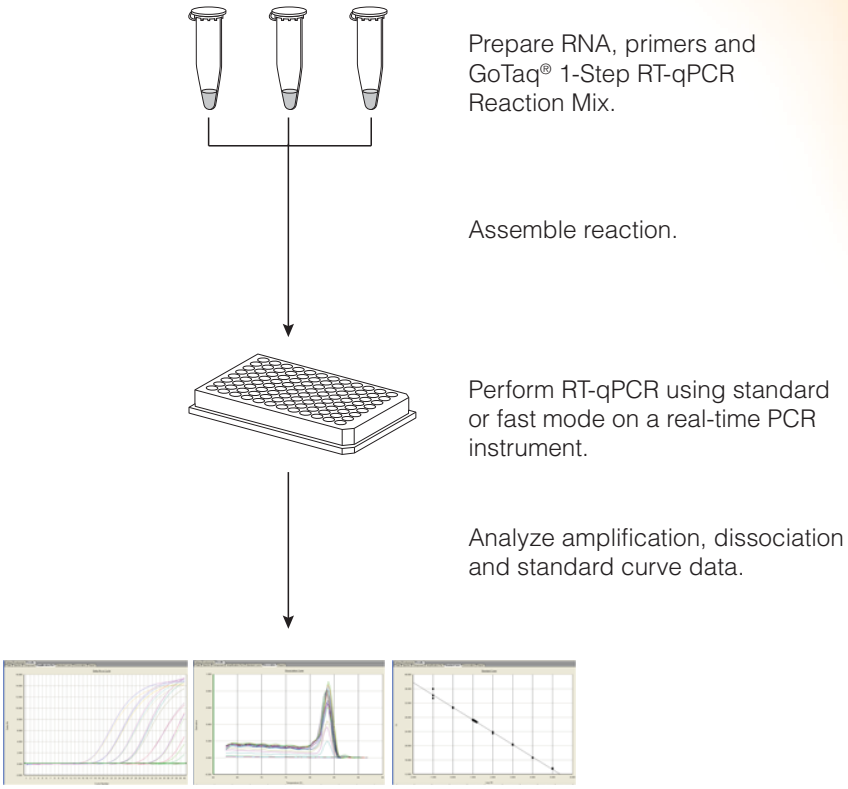

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Table 1. General Thermocycler Program.

Stage	# of Cycles	Program in Standard or Fast Mode
1. Reverse transcription	1	≥37°C for 15 minutes
2. RT inactivation/Hot-start activation	1	95°C for 10 minutes
3. 3-Step qPCR: a. Denature b. Anneal/Collect data c. Extend	40	95°C for 10 seconds 60°C for 30 seconds 72°C for 30 seconds
4. Dissociation	1	60–95°C

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Protocol (continued)

1. Program a real-time instrument for standard or fast mode one-step RT-qPCR (Table 1).
2. Thaw the components of the GoTaq® 1-Step RT-qPCR System, the RNA templates and the primer pair on ice, at room temperature or at 37°C. Immediately mix each thawed component thoroughly. If using a vortex mixer, mix at **low speed** to minimize aeration. Keep thawed reagents on ice.
3. Prepare:
   a. RNA samples (total RNA, mRNA, viral RNA or transcript RNA [500fg–100ng]) in water or other qPCR-compatible diluent.
   b. Standards and controls.
   c. Primer pair: 1X concentration is approximately 50–300nM.
4. Combine reaction components (Table 2) in a nonstick, sterile tube on ice. Mix gently after each addition. Carefully pipet reaction volumes to plate on ice.
5. Transfer plate from ice into the preprogrammed instrument. Start the run immediately.
6. When the run is complete, collect the data and analyze the results.

Table 2. GoTaq® 1-Step RT-qPCR Reaction Mix.

Component	Volume per 20µl Reaction	Volume per 50µl Reaction	Final Concentration in Reaction
GoTaq® qPCR Master Mix, 2X	10µl	25µl	1X
Forward Primer, 10X	2µl	5µl	50–300nM
Reverse Primer, 10X	2µl	5µl	50–300nM
GoScript™ RT Mix for 1-Step RT-qPCR, 50X **or** Nuclease-Free Water for Minus-RT Control	0.4µl	1.0µl	1X
RNA Template (500fg–100ng) **or** Nuclease-Free Water for No-Template Control	4µl	10µl	variable
Optional: $MgCl_2$, 25mM*	__µl	__µl	≥2mM
Optional: CXR Reference Dye, 30µM**	__µl	__µl	≥33nM
Nuclease-Free Water	to 20µl	to 50µl	—

*To supplement the $MgCl_2$ provided in Master Mix.

**Guidelines for addition of CXR Reference Dye (30µM) to the reaction mix to achieve a final concentration of 0.5µM:
31µl per 100-reaction batch for 20µl reactions or
78µl per 100-reaction batch for 50µl reactions

Detailed protocols and instructions for a variety of instruments can be found in the *GoTaq® 1-Step RT-qPCR System Technical Manual* #TM355, available at: **www.promega.com/resources/protocols/**

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