GoTaq® 2-Step RT-qPCR System

INSTRUCTIONS FOR USE OF PRODUCT A6010.

Quick PROTOCOL

For more information, see the *GoTaq® 2-Step RT-qPCR System Technical Manual* #TM337, available at: **www.promega.com/tbs**

Protocol

Prepare RNA and Reverse Transcription Primer

1. Combine RNA and reverse transcription primer in reaction tube or well of multiwell plate on ice. Close or seal tightly.

Component	Volume
RNA (up to 5µg/reaction)	__µl
Primer [Oligo(dT)$_{15}$ Primer and/or	1µl
Random Primer or	1µl
gene-specific primer]	__µl
Nuclease-Free Water (up to a final volume of 10µl)	__µl
Final Volume	**10µl**

2. **Optional:** Denature the RNA and reverse transcription primer at 70°C for 5 minutes. Chill at 4°C for 5 minutes.
3. Store RNA and reverse transcription primer on ice prior to adding the GoScript™ Reaction Mix.

Synthesize cDNA with GoScript™ Reverse Transcriptase

4. Prepare the GoScript™ Reaction Mix on ice by adding the components in the order listed below.

Component	GoScript™ Reaction Mix	Minus-Reverse Transcriptase Reaction Mix
Nuclease-Free Water (to a final volume of 10µl)	1.5µl	2.5µl
GoScript™ 5X Reaction Buffer	4µl	4µl
$MgCl_2$, 25mM	2µl	2µl
PCR Nucleotide Mix, 10mM	1µl	1µl
Recombinant RNasin® Ribonuclease Inhibitor	0.5µl	0.5µl
GoScript™ Reverse Transcriptase	1µl	0µl
Final Volume	**10µl**	**10µl**

5. Combine the GoScript™ Reaction Mix with the RNA and reverse transcription primer in reaction tubes or multiwell plate.

Component	Reverse Transcriptase Reaction
GoScript™ Reaction Mix or Minus-Reverse Transcriptase Reaction Mix	10µl
RNA and reverse transcription primer prepared in Steps 1–3	10µl
Final Volume	**20µl**

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Protocol (continued)

Synthesize cDNA with GoScript™ Reverse Transcriptase (continued)

6. Synthesize the cDNA using the reaction conditions below.

Step	Temperature	Time
Anneal (Optional)	25°C	5 minutes
Extend	42°C	1 hour
Inactivate	70°C	15 minutes

Note: The annealing, extension and inactivation conditions can be modified. See Technical Manual #TM337 for details.

7. Store cDNA at 4°C or on ice for immediate analysis. Alternatively, store the cDNA at –20°C until use.

Quantify cDNA with GoTaq® qPCR Master Mix

8. Prepare diluted cDNA and reference standards for qPCR in nuclease-free water.
9. Carefully add 10µl of each diluted or undiluted cDNA or reference standard to the appropriate wells of the reaction plate. For no-template control reactions, add 10µl of nuclease-free water. Cover plate and store on ice.
10. Prepare GoTaq® qPCR Reaction Mix at room temperature or on ice by adding the components in the order specified below. Mix gently. Do **not** vortex. Minimize exposure to light.

Component	Volume
GoTaq® qPCR Master Mix, 2X	25µl
Nuclease-Free Water (to a final volume of 40µl)	__µl
Forward and reverse qPCR primers[1]	__µl
Final Volume	**40µl**

[1]A range of primer concentrations can be used. See Technical Manual #TM337 for details.

Notes

1. See *GoTaq® 2-Step RT-qPCR System Technical Manual* #TM337 for a list of instruments that require addition of the CXR Reference Dye.
2. Some instruments such as the BioRad instruments require addition of a normalization dye (e.g., fluorescein).

11. Combine the 40µl of GoTaq® qPCR Reaction Mix with each 10µl of cDNA template, reference standards or water (no-template control) in multiwell plates at room temperature or on ice. Final qPCR volume is 50µl. Centrifuge briefly.
12. Program the real-time PCR instrument for standard or fast qPCR. Standard conditions are listed below:

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

13. Place the reaction plate in the real-time PCR instrument, and press start.

Detailed protocols and instructions can be found in the *GoTaq® 2-Step RT-qPCR System Technical Manual* #TM337, available online at: **www.promega.com/tbs**

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