

Using GoTaq® qPCR Master Mix

The protocol for a 20µl reaction is outlined below. Component volumes may be scaled as appropriate. This protocol assumes that 20% of the reaction volume is DNA template (e.g., 4µl of DNA template added to 16µl of reaction mix). If the volume of DNA template is more or less than 4µl, adjust the volume of Nuclease-Free Water accordingly so that the final reaction volume is 20µl.

Assembling the Reaction Mix

1. Prepare the reaction mix by combining GoTaq® qPCR Master Mix, Nuclease-Free Water and primers in the order listed. See Notes a and b.

Component	Volume	Final Concentration
GoTaq® qPCR Master Mix (2X)	10µl	1X
Forward Primer (20X)	1µl	200nM–1µM
Reverse Primer (20X)	1µl	200nM–1µM
Supplemental CXR Reference Dye (if required)	0.2µl per reaction	300nM
Nuclease-Free Water	to a final volume of 16µl	

Notes:

- a. See the *GoTaq® qPCR Master Mix Technical Manual #TM318* for a list of instruments that require adding CXR Reference Dye.
 - b. Some instruments such as the BioRad instruments require adding a normalization dye (e.g., fluorescein).
2. Carefully pipet 16µl of reaction mix into each reaction well.
 3. Add 4µl of experimental template, standard or negative control to the reaction mix dispensed in Step 2.
 4. Seal the plate, and centrifuge at low speed for 1 minute.
 5. Program the thermal cycler with the desired thermal cycling conditions as per the manufacturer's instructions.
 6. Place the plate in thermal cycler, and press **Start**.

When the run is complete, analyze the data.

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Additional protocol information is in Technical Manual #TM318, available online at: www.promega.com