GoTaq® Probe qPCR Master Mix

Instructions for Use of Products A6101 and A6102.

Promega

Quick Protocol

GoTaq® Probe qPCR Master Mix 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 of GoTaq® Probe qPCR Master Mix at either a "low dye" or "high dye" concentration. Refer to the *GoTaq® Probe qPCR System Technical Manual* #TM378, Section 4.A, for detailed information.

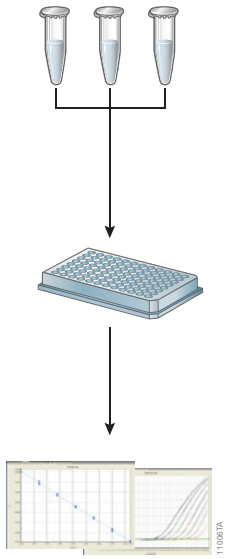
Preparation of GoTaq® Probe qPCR Reaction Mix

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 DNA 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 DNA	2–5µl	≤250ng
Nuclease-Free Water	to a final volume of 20µl	

5. Add reaction mix (minus DNA template) to each PCR tube or well of an optical-grade PCR plate.
6. Add DNA 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.



Prepare GoTaq® Probe qPCR Reaction Mix by combining primers, probe and GoTaq® Probe qPCR Master Mix.

Assemble reaction.

Perform qPCR using standard or FAST mode on a real-time PCR instrument.

Analyze amplification and standard curve data.

GoTaq® Probe qPCR Master Mix 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
GoTaq® DNA 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
GoTaq® DNA Polymerase activation	1	95°C	2 minutes
Denaturation	40	95°C	3 seconds
Annealing/Extension		60°C	30 seconds

Additional protocol information is in Technical Manual #TM378, available online at: **www.promega.com**

