GoTaq® Probe qPCR Master Mix

INSTRUCTIONS FOR USE OF PRODUCTS A6101 AND A6102.

Quick PROTOCOL

For more information, see the *GoTaq® Probe qPCR Master Mix Technical Manual* #TM378, available at: **www.promega.com/protocols**

GoTaq® Probe qPCR Master Mix Protocol

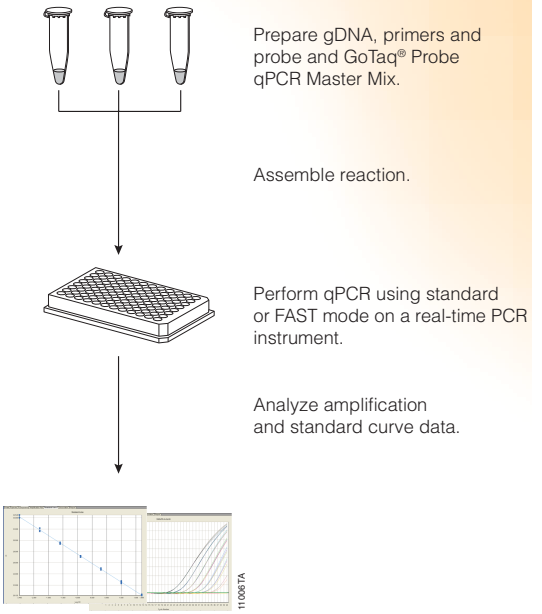
Addition of CXR Reference Dye to GoTaq® qPCR Master Mix (Optional)

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 Amplifications

The GoTaq® Probe qPCR Master Mix uses a hot-start chemistry, allowing reaction setup to be done at room temperature.

1. Thaw the GoTaq® Probe qPCR Master Mix and the Nuclease-Free Water. **Do not** thaw the Master Mix at temperatures above room temperature.
2. Vortex the GoTaq® Probe qPCR Master Mix for 3–5 seconds.
3. Determine the number of reactions to prepare, plus negative controls, then increase the number by 1–2 to compensate for pipetting error.



Component	Volume	Final Reaction Concentration
GoTaq® Probe qPCR Master Mix (2X)	10µl	1X
Forward primer (20X)	1µl	900nM
Reverse primer (20X)	1µl	900nM
Hydrolysis probe (20X)	1µl	250nM
Template DNA	2–5µl	≤250ng
Nuclease-Free Water	To a final 20µl reaction volume	

4. Prepare the reaction (minus DNA template) by combining the GoTaq® Probe qPCR Master Mix, PCR primers, hydrolysis probe and Nuclease-Free Water. Vortex.
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 sample reactions.
7. Seal the tubes or plates, centrifuge briefly to collect components to the bottom of the tubes or wells. Protect from light. Samples are now ready for thermal cycling.

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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® 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® polymerase activation	1	95°C	2 minutes
Denaturation	40	95°C	3 seconds
Annealing/Extension		60°C	30 seconds

Detailed protocols and instructions can be found in the *GoTaq® Probe qPCR Master Mix Technical Manual* #TM378, available online at: **www.promega.com/protocols**

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