GoTaq® Probe qPCR: A Real-Time PCR Master Mix Optimized for Hydrolysis Probe Assay

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Abstratct

The **GoTaq® Probe qPCR Master Mix** is a ready-to-use, 2X master mix for qPCR using hydrolysis probe detection, and is designed for sensitive detection and quantification over a broad range of DNA or RNA targets in the presence of a wide range of PCR inhibitors. Rapid hot-start activation and processive enzymes makes these mixes compatible with any real-time PCR.

The **GoTaq® Probe 2-Step RT-qPCR** facilitates detection and relative quantification of RNA expression levels via a two-step RT-qPCR method. The GoScript™ Reverse Transcription System enables efficient synthesis of first-strand cDNA which may be added directly to downstream qPCR reactions.

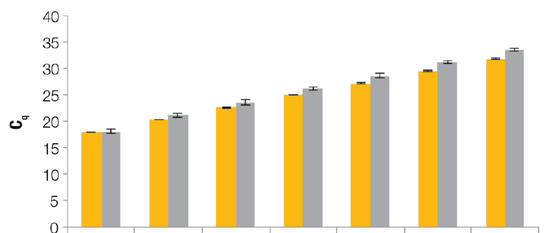
The **GoTaq® Probe 1-Step RT-qPCR with dUTP** is optimized for detection and relative quantification of RNA expression levels using a one-step RT-qPCR method, combining GoScript™ Reverse Transcriptase and GoTaq® Probe qPCR Master Mix in single-step real-time amplification reactions.

The master mix does not contain a reference dye; a separate tube of carboxy-X-rhodamine (CXR) reference dye is included.

Features of the GoTaq® Probe qPCR Master Mix

- Designed to provide resistance to a wide range of PCR inhibitors.
- Uses antibody-mediated hot-start chemistry, allowing reaction setup to be performed at room temperature.
- Employs rapid hot-start activation and processive enzymes, making it compatible with both standard and fast instrument cycling programs.

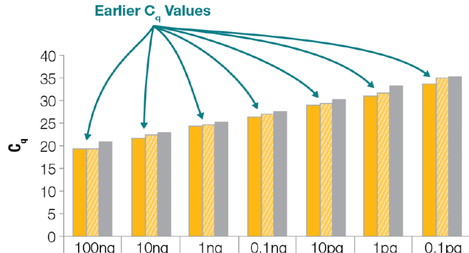
Sensitivity - Detection of picograms levels of RNA input



	100ng	20ng	4ng	0.8ng	160pg	32pg	64pg
Promega	18.0	20.4	22.6	25.1	27.3	29.6	31.8
Vendor L	18.2	21.2	23.6	26.1	28.7	31.2	33.6

Amplification curve for GAPDH detection from human pancreas RNA using the GoTaq® Probe 2-Step RT-qPCR System. A series of fivefold serial dilutions of human pancreas cDNA was used to measure GAPDH gene expression using the GoTaq® Probe 2-Step RT-qPCR System and a competing product (Vendor L). The GoTaq® Probe 2-Step RT-qPCR System shows earlier Cq values (>0.2) for all samples. Two microliters of human pancreas RNA was reverse transcribed using the GoScript™ Reverse Transcriptase supplied in the kit followed by qPCR with GoTaq® Probe qPCR Master Mix.

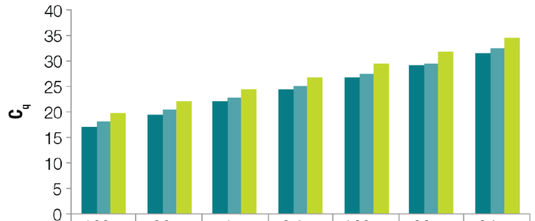
Versatility - Works with both standard and fast thermal-cycling conditions



	100ng	10ng	1ng	0.1ng	10pg	1pg	0.1pg
Promega—Standard	19.3	21.7	24.3	26.3	29.0	31.1	33.8
Promega—FAST	19.2	22.2	24.7	26.9	29.3	31.7	34.9
Vendor L—FAST	20.9	23.1	25.4	27.8	30.3	33.2	35.5

GoTaq® Probe qPCR Master Mix shows comparable results when GAPDH is amplified from human pancreas cDNA using standard and fast thermal-cycling conditions. Earlier Cq values were observed for both standard and fast thermal-cycling conditions when GoTaq® Probe qPCR Master Mix was compared to a competing product (Vendor L).

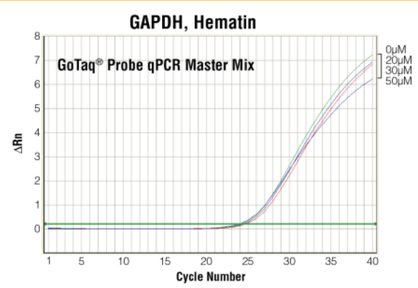
Compatibility - Suitable for PrimeTime®, TaqMan®, and Molecular Beacon probe chemistries



	100ng	20ng	4ng	0.8ng	160pg	32pg	64pg
PrimeTime®	17.1	19.2	21.9	24.2	26.5	28.9	31.2
TaqMan®	17.9	20.4	22.6	24.9	27.2	29.5	32.2
Molecular Beacon	19.6	22.1	24.4	26.8	29.2	31.7	34.3

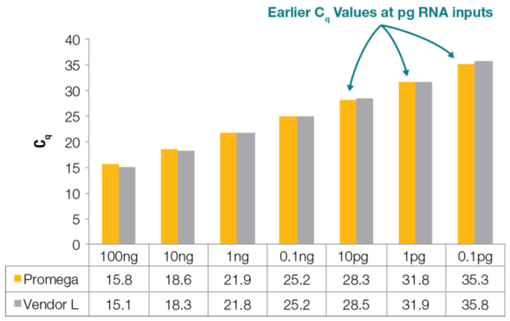
GoTaq® Probe 2-Step RT-qPCR System works with a variety of probe assay chemistries. A series of 7 fivefold dilutions of human heart RNA was analyzed for GAPDH gene expression using PrimeTime®, TaqMan®, and Molecular Beacon hydrolysis probe chemistries and the GoTaq® Probe 2-Step RT-qPCR System. All sample dilutions generated results with all chemistries tested using the GoTaq® Probe 2-Step RT-qPCR System.

Robustness - Resistant to a wide range of qPCR inhibitors



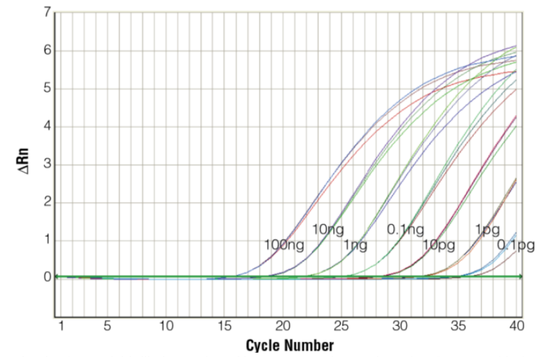
0–50µM of Hematin added to qPCR reactions amplifying GAPDH from human liver RNA. RNA was converted to cDNA with GoScript™ Reverse Transcription System. GoTaq® was robust enough to amplify all samples containing Hematin.

Dynamic Range – RNA detection from 100 ng down to 1pg



	100ng	10ng	1ng	0.1ng	10pg	1pg	0.1pg
Promega	15.8	18.6	21.9	25.2	28.3	31.8	35.3
Vendor L	15.1	18.3	21.8	25.2	28.5	31.9	35.8

Cq values for 6 × tenfold dilution series of human control RNA detecting GAPDH in a 20µl 1-step RT-qPCR reaction comparing GoTaq® Probe 1-Step RT-qPCR System to a 1-Step RT-qPCR master mix from Vendor L. GoTaq® Probe 1-Step RT-qPCR System gave earlier Cq values with the pg of RNA samples showing greater sensitivity.



Amplification curve for the 6 × tenfold dilution series of human control RNA detecting GAPDH with the GoTaq® Probe 1-Step RT-qPCR System. The qPCR reaction efficiency was calculated to be 102.2% with an r2 of 0.998.

Summary

GoTaq® Probe qPCR and RT-qPCR Master Mixes are optimized for qPCR assays in the hydrolysis probe detection format and offers:

- Optimized formulation for a variety of PCR applications
- Easy room-temperature setup for automated and high-throughput detection
- Sensitive detection on most real-time instruments
- Versatility with both fast and standard cycling methods
- Resistant to a wide variety of PCR inhibitors
- Compatible with multiple probe chemistries
- Robust, highly efficient, full-length cDNA synthesis in the presence of inhibitors
- Dynamic range >6 orders of magnitude
- Backed by the Promega PCR Performance Guarantee