

USB® VeriQuest™ Fast Probe qPCR Master Mix, No Reference Dye (2X)

Applications:

- Amplification from genomic DNA or cDNA input
- Gene expression validation

Features:

- Uses fast mode thermal cycling conditions for results in one-third of the time when compared to standard run protocols
- One-tube master mix—just add template, primers and water
- Reproducibility and consistency over a broad dynamic range: 8 orders of magnitude linear detection range (Fig. 1)
- High specificity with exceptional performance on challenging GC-rich regions
- High sensitivity and precision with limited targets (Fig. 2)
- Stable at room temperature for 72 hours in a pre-assembled reaction (Fig. 3)
- Contains dUTP and Uracil-DNA Glycosylase (UDG or UNG) for carry-over contamination prevention
- Compatible with a wide variety of real-time PCR instruments

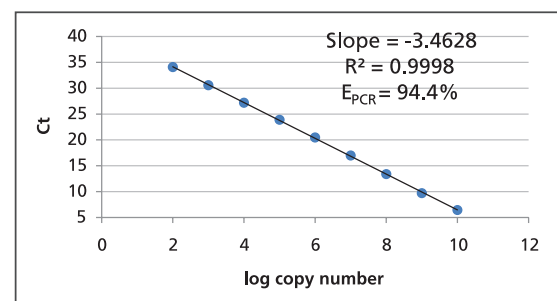
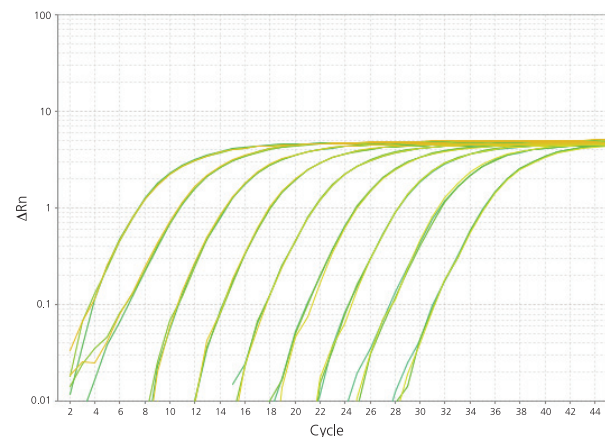
USB VeriQuest Fast Probe qPCR Master Mix, No Reference Dye is optimized for SYBR® Green detection on all instruments that utilize ROX™ as a passive reference dye. This master mix can be run using Fast mode cycling protocols, providing results in one-third of the time when compared to standard real-time PCR reagents. The 2X ready-to-use master mix contains hot start VeriQuest Taq DNA Polymerase, MgCl₂, ultrapure nucleotides with an optimized dUTP:dTTP ratio and Uracil-DNA Glycosylase (UDG or UNG) in a proprietary reaction buffer. The hot start Taq polymerase has no polymerase activity prior to the initial heat activation step which allows reaction assembly at room temperature. The mix offers higher specificity and sensitivity, virtually eliminating non-specific primer amplification which can negatively affect the efficiency and accuracy of the data (Fig. 1). Since the mix contains dUTP and UDG, carry-over contamination prevention can be performed prior to amplification. VeriQuest Fast Probe qPCR Master Mix, No Reference Dye offers the same sensitivity and specificity found with our standard mode mixes with results in a fraction of the time.

VeriQuest Fast Probe Master Mix, No Reference Dye shows exceptional efficiency and high specificity on challenging templates such as high GC- and AT-rich regions. The master mix is also capable of duplex target detection without compromising the integrity of the data (Fig. 4). The optimized formulation ensures no sacrifice in quality with increased speed.

The sensitivity of the master mix allows for discrimination from a 1.33-fold difference in gene target amount detected. In Figure 2 a 1.33 to 10-fold dilution series of 10 ng to 1 ng of cDNA reverse-transcribed from HeLa total RNA were amplified with an efficiency of >98.8%.

VeriQuest Fast Probe qPCR Master Mix, No Reference Dye is highly stable and easy to work with. It remains stable at room temperature for 72 hours in a pre-assembled reaction and can be stored at 4°C for convenient handling. The mix also allows for room temperature reaction set-up. The speed and stability make VeriQuest Fast Probe qPCR Master Mix, No Reference Dye ideal for high-throughput handling.

Fig. 1. Linear detection range



Linear detection range of VeriQuest Fast Probe qPCR Master Mix. Amplification plot and standard curve from real-time PCR for a dilution series of a synthetic target with starting amounts of 10¹⁰ copies amplified in four replicate reactions using the ABI 7500 Real-Time PCR System and GAPDH primers. Reaction performed using fast mode cycling with 5 minutes activation at 95°C. The amplification process was linear over eight orders of magnitude.

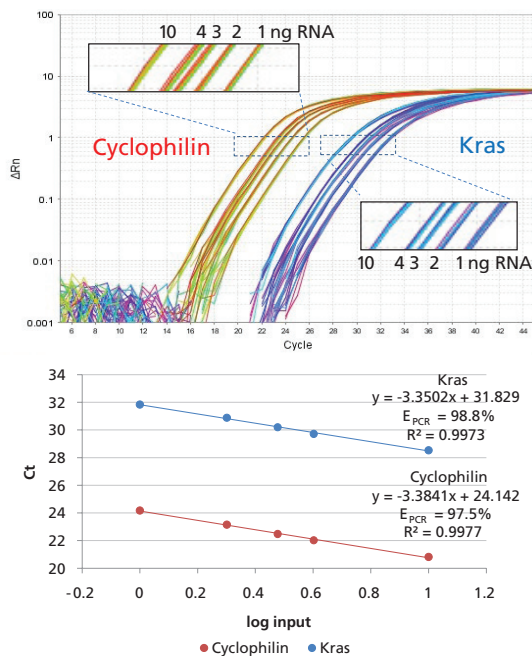
Components:

75685	100 reactions	1 ml
	500 reactions	5 ml
	1,000 reactions	2 x 5 ml
	2,500 reactions	5 x 5 ml
	5,000 reactions	10 x 5 ml

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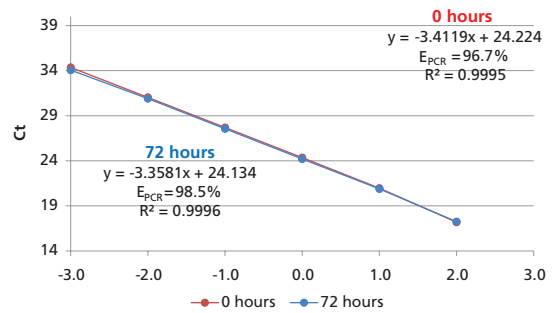
Product Code	Pack Size
75685	100 reactions
	500 reactions
	1,000 reactions
	2,500 reactions
	5,000 reactions

Fig. 2. High sensitivity and precision



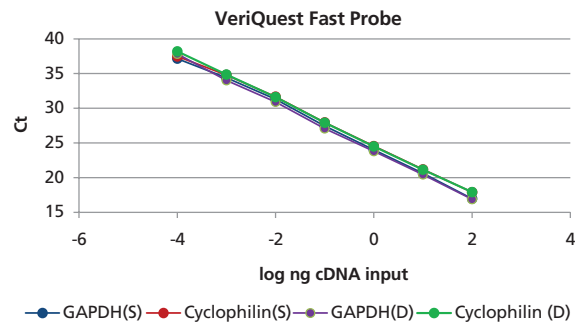
High sensitivity and precision in limited target quantification. Amplification plot (bottom) and standard curve (top) from real-time PCR for a 1.33 to 10-fold dilution series of 10 ng to 1 ng of cDNA reverse-transcribed from HeLa total RNA.

Fig. 3. VeriQuest Fast Probe qPCR Master Mix stability



GAPDH was detected from pre-assembled PCR reactions incubated at room temperature for 72 hours. Results were compared to the freshly prepared mix (0 hours).

Fig. 4. Single-target and duplex PCR amplification



USB VeriQuest Fast Probe Mix			
	slope	E_{PCR}	R^2
GAPDH(S)	-3.4039	96.7%	0.9983
Cyclophilin(S)	-3.3229	100.0%	0.9986
GAPDH(D)	-3.4690	94.2%	0.9996
Cyclophilin(D)	-3.4069	96.6%	0.9998
Fast cycling conditions			
USB VeriQuest Probe Mix			
	slope	E_{PCR}	R^2
GAPDH(S)	-3.4507	94.9%	0.9996
Cyclophilin(S)	-3.4116	96.4%	0.9999
GAPDH(D)	-3.3623	98.3%	1.0000
Cyclophilin(D)	-3.3996	96.9%	1.0000
Standard cycling conditions			

VeriQuest Fast Probe qPCR Master Mix single-target and duplex PCR amplification of Cyclophilin Fam-BHQ-1® probe and GAPDH Cy5-BHQ-1 probe. Single-target (S) and duplex reactions (D).

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