PrimeScript™ RT Master Mix (Perfect Real Time) (Cat.# RR036A)

Application: The effect of reverse transcription reaction time on the amount of cDNA synthesized: Efficient cDNA synthesis in as little as 15 minutes

PrimeScript RT Master Mix (Perfect Real Time) (Cat.# RR036A) is a reverse transcription reagent kit designed to generate template cDNA for use in real time RT-PCR. The kit uses PrimeScript RTase, which allows efficient cDNA synthesis in just 15 minutes.

Methods:

1. Reverse Transcription Reaction

Reagent:	PrimeScript RT Master Mix (Perfect Real Time) (Cat.# RR036A)
Template:	Human Placenta total RNA (2 pg–2 µg) and sterile dH_2O
Reaction volume:	20 µl
Reaction conditions:	37°C for 15, 30, or 60 min. → 85°C for 5 sec. → 4°C hold

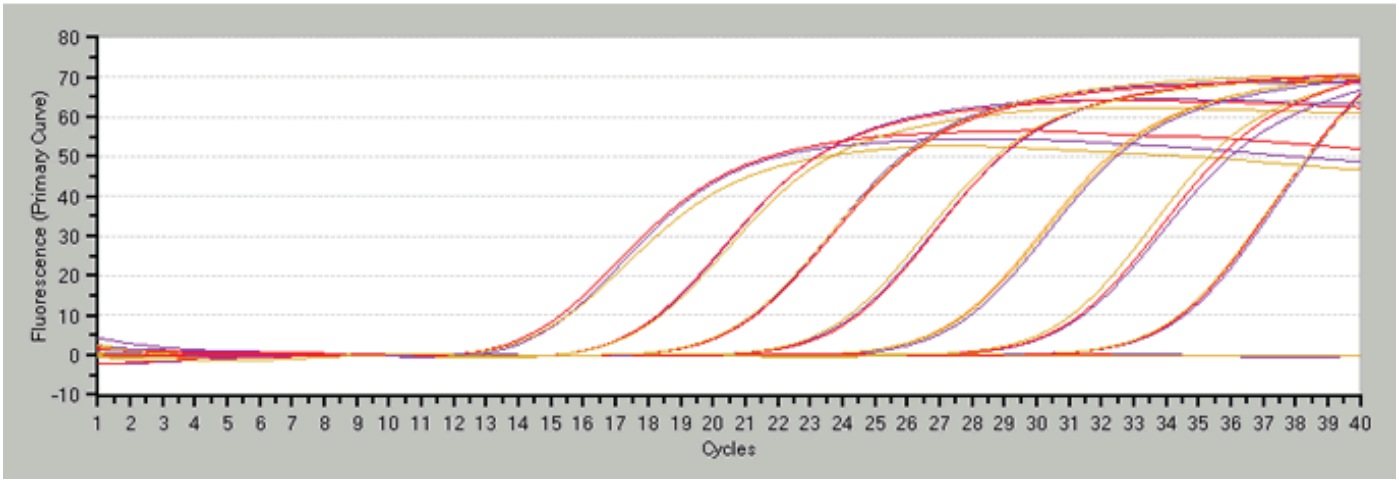
2. Real Time PCR

Reagent:	SYBR® *Premix Ex Taq* II (Perfect Real Time) (Cat.# RR820A)
Template:	2 µl of synthesized cDNA
Reaction volume:	25 µl
Reaction conditions:	Thermal Cycler Dice Real Time System (standard protocol)

Results:

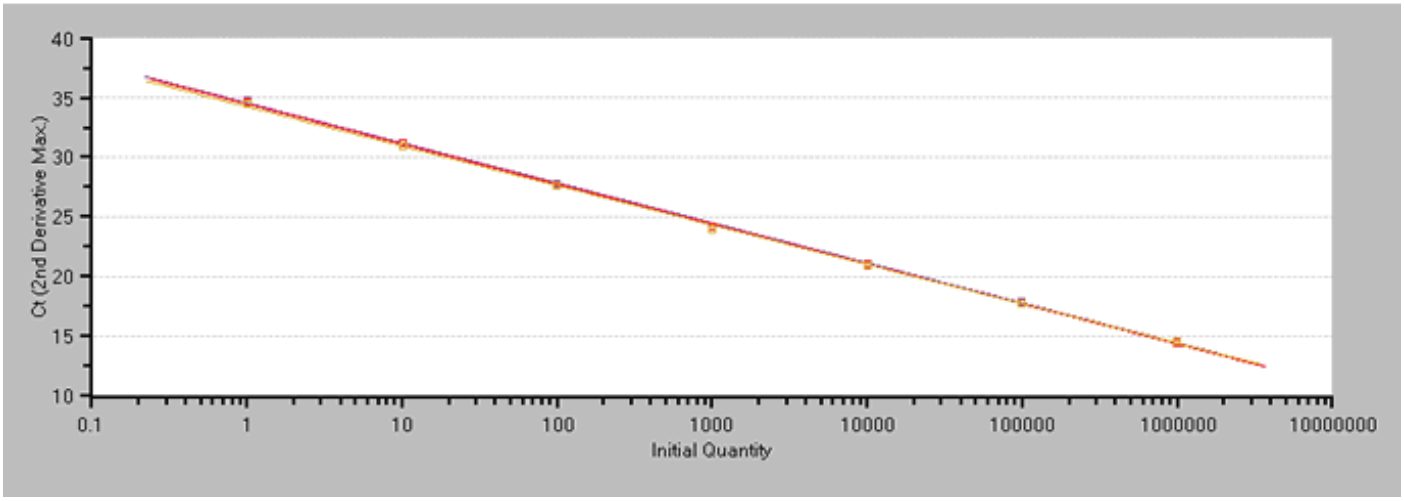
Using the amounts of template RNA indicated, cDNA reactions were allowed to proceed for 15, 30, or 60 min. The amplification curves and standard curve obtained after real time PCR are plotted in Figure 1, with each color corresponding to a different cDNA synthesis time.

Amplification curve



Application: The effect of reverse transcription reaction time on the amount of cDNA synthesized: Efficient cDNA synthesis in as little as 15 minutes (cont.)

Standard curve



	Time (min.)	R^2	Efficiency (%)	Standard Curve
Violet	15	0.999	99.0	Y = −3.346 x LOG(X)+34.52
Red	30	1.000	98.9	Y = −3.349 x LOG(X)+34.46
Yellow	60	0.999	100.9	Y = −3.301 x LOG(X)+34.24

Conclusions:

With PrimeScript RT Master Mix, reactions can proceed with equivalent efficiency over a wide range of template RNA concentrations for reaction times ranging from 15 to 60 min.