PRIMETIME™ ONE-STEP RT-qPCR MASTER MIX

Reagent	Size	Catalog #
PrimeTime One-Step RT-qPCR Master Mix*	1 x 1 mL	10007065
	1 x 5 mL	10007066
	5 x 5 mL	10007067
	20 x 25 mL	10007068
Nuclease-Free Water	10 x 2 mL	11-04-02-01
	300 mL	11-05-01-14

* Separate tubes of reference dye are included with all sizes, except the 20 x 25 mL size. (If this size is needed, go to http://www.idtdna.com/ContactUs.)

Overview

The PrimeTime One-Step RT-qPCR Master Mix is a ready-to-use, 2X concentrated master mix that is designed for use in probe-based, real-time quantitative PCR. The PrimeTime One-Step RT-qPCR Master Mix contains an antibody-mediated hot-start DNA polymerase, reverse transcriptase, dNTPs, $MgCl_2$, enhancers, and stabilizers.

Note: The PrimeTime One-Step RT-qPCR Master Mix is shipped on dry ice. Upon receipt, store at –15 to –30°C in a constant temperature (non–frost free) freezer. Avoid repeated cycles of freezing and thawing.

Guidelines

- Overall, the time between the addition of template and starting the qPCR run should be minimized.
- Ideally, qPCR plates should be setup on ice, or at 4°C. Depending on the stability of the RNA template, plates containing reaction mix and template typically can be stored at 4°C for up to 8 hours before the qPCR run without negatively affecting performance of the master mix.
- If plates are setup at room temperature (20–25°C), the qPCR run should be initiated *no more than 30 minutes* after template addition.

Protocol

Prepare reagents

1. Thaw the following reagents on ice:
   - PrimeTime One-Step RT-qPCR Master Mix
   - Reference dye
   - Primer and probe solutions
   - Template RNA
2. Gently invert 2–3 times to mix reagents thoroughly.
3. Quickly centrifuge to collect solutions at the bottom of tubes.

Add reference dye

Add the appropriate amount of reference dye, **if needed** (refer to Table 1), to the PrimeTime One-Step RT-qPCR Master Mix (Table 2).

Table 1. Reference dye concentration levels required by various PCR systems.

PCR System*	Reference dye		
	High	Low	None
7900HT Fast and 7300 Real-Time PCR System (Thermo Fisher Scientific)	X		
StepOne™ and StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific)	X		
Mx3005P™ and Mx4000P™ qPCR System (Agilent)		X	
7500 Real-Time PCR System (Thermo Fisher Scientific)		X	
Viia™ 7 Real-Time PCR System (Thermo Fisher Scientific)		X	
QuantStudio™ Flex Systems (Thermo Fisher Scientific)		X	
CFX, iQ™, and Opticon™ Real-Time PCR Detection Systems (Bio-Rad)			X
LightCycler® Real-Time PCR Systems (Roche)			X

* For instruments not listed, check with the manufacturer.

Table 2. Amount of reference dye to add to PrimeTime One-Step RT-qPCR Master Mix.

	Volume of 25 µM dye (µL)	
	High	Low
To a 1 mL stock vial of master mix, add:	40	4
To a 5 mL stock vial of master mix, add:	200	20
To a 25 mL stock vial of master mix, add:	1000	100

Prepare the Reaction Mix

1. Determine the total number of reactions in your experiment, including replicates, controls (e.g., no template control, positive control), and 1–3 additional reactions to account for pipetting errors.
2. Combine all components **except for the RNA template** (Table 3) and invert 4–6 times to mix.

Notes:

- RNA template will be added to the Reaction Mix in the qPCR plate in the next section, Add RNA template.
- The volumes provided in Table 3 are *per reaction*; calculate the final component volumes by multiplying each volume by the total number of reactions.

Table 3. Reaction mixes.

PrimeTime qPCR Assay reactions (Premixed primers and probe)			
Component	Final conc. or amount	Volume per 20 µL reaction	Volume per 10 µL reaction
PrimeTime One-Step RT-qPCR Master Mix (2X)	1X	10 µL	5 µL
PrimeTime qPCR Assay* (20X)	1X	1 µL	0.5 µL
RNA template (*do not add, yet*)	20 pg to 200 ng	2–5 µL	2–4.5 µL
Nuclease-Free Water		Up to 20 µL	Up to 10 µL
qPCR using separate primers and probe			
Component	Final conc. or amount	Volume per 20 µL reaction	Volume per 10 µL reaction
PrimeTime One-Step RT-qPCR Master Mix (2X)	1X	10 µL	5 µL
Forward and reverse primers	250–1000 nM each	Variable	Variable
Probe(s)	150–250 nM each	Variable	Variable
RNA template (*do not add, yet*)	20 pg to 200 ng	2–5 µL	2–4 µL
Nuclease-Free Water		Up to 20 µL	Up to 10 µL

* To order, visit www.idtdna.com/PrimeTime.

3. Briefly centrifuge, then dispense equal aliquots of the Reaction Mix into the wells of a qPCR plate that is compatible with your real-time PCR instrument.

Add RNA template

1. Add 20 pg to 200 ng of RNA template or controls to the wells of the qPCR plate that contain Reaction Mix.

Note: Now your total volume should be either 20 or 10 µL, depending on your chosen reaction volume (See Table 3).

2. Seal the qPCR plate with optically transparent film.
3. Gently agitate to ensure thorough mixing, then briefly centrifuge to remove air bubbles and collect the reaction at the bottom of the wells.

Set up cycling program

Program the appropriate PCR cycling protocol on your real-time PCR instrument (refer to Table 4).

Important: For most assays, 50°C for 15 minutes provides ideal conditions for reverse transcriptase extension (Table 4).

The reverse transcriptase extension temperature may range between 42–60°C, and the time may be as long as 1 hour. However, higher temperatures and longer times may result in lower reverse transcriptase stability and activity.

Table 4. Cycling protocol.

Step	Cycles	Temperature (°C)	Fast cycling	Standard cycling
Reverse transcription	1	50	15 min	15 min
Polymerase activation*	1	95	3 min	3 min
Amplification:				
Denaturation	35–45	95	5 sec	15 sec
Annealing/extension†		60	30 sec	1 min
Hold, if needed	1	4	Up to 24 hr	Up to 24 hr

* **Important:** Do not change the polymerase activation conditions.
† This is a general starting point. The annealing/extension temperature or time may need to be adjusted based on which primer sequences you use.

Run PCR

Place the plate in the real-time PCR instrument and start the cycling program.

> Find safety data sheets (SDSs) and certificate of analysis (COAs) for IDT products at www.idtdna.com/1-step-master-mix.

> For additional information or assistance, go to http://www.idtdna.com/ContactUs.