PrimeTime® One-Step 4X Broad-Range Master Mix

Product	Quantity*	Catalog #
PrimeTime One-Step 4X Broad-Range Master Mix	1 x 1 mL	10011744
	5 x 1 mL	10011745
	2 x 5 mL	10011746
	5 x 5 mL	10011747
	25 x 10 mL	10011748
	20 x 25 mL	Contact Us

* Separate tubes of reference dye and Direct Amplification Enhancer are included with all orders, except for the 25 x 10 mL and 20 x 25 mL sizes (Contact us to order these products).

Note: Visit https://www.idtdna.com/pages/support/guides-and-protocols to verify that you are using the most current version of this protocol.

Contents

PrimeTime One-Step 4X Broad-Range Master Mix is a ready-to-use, 4X concentrated master mix that is designed for use in probe-based, real-time quantitative PCR. PrimeTime One-Step 4X Broad-Range Master Mix contains a hot-start reverse transcriptase, a hot-start DNA polymerase, dNTPs, $MgCl_2$, enhancers, and stabilizers.

In addition, an enhancer solution is provided as an optional additive to neutralize PCR inhibitors including nucleases in crude samples.

A reference dye is provided as a separate component, making this master mix compatible for use on both reference dye-dependent and independent instrument systems.

Storage

PrimeTime One-Step 4X Broad-Range Master Mix is shipped on dry ice. Upon receipt, store as follows:
–15 to –30°C in a constant temperature (non–frost free) freezer until the end of the month indicated in the expiration date, or 2 to 8°C for 1 month.

Notes:

- Avoid repeated freezing and thawing.
- Avoid prolonged exposure of reference dye to light.
- Product will arrive solid and should be thawed at 4°C or on ice. Product should remain liquid in the –20°C freezer. However, temperature fluctuations may cause the product to freeze solid. This is normal.

Prepare the Reaction Mix

1. Determine the number of reactions needed for your experiment, including replicates, controls (no template control; positive control), and 1–3 additional reactions to adjust for pipetting differences.
2. Combine all components, **except for the DNA/RNA template** (Table 1), and thoroughly mix.

Notes:

- DNA/RNA template will be added to the Reaction Mix in the qPCR plate in the Add DNA/RNA template section.
- The volumes provided in Table 1 are per reaction; calculate the final component volumes by multiplying each volume by the total number of reactions.
- Plates pre-aliquoted with master mix (without DNA/RNA template) can be stored at 4°C for up to 8 hours.
- If not pre-aliquoted, while aliquoting and loading reaction mix and template, it is critical that the reaction mix and PCR plate are kept on ice or on a 4°C block until it is ready to be loaded onto the qPCR instrument.

Table 1. Reaction mixes.

Standard RT-qPCR reaction using purified RNA			
Component	Final concentration or amount	Volume per 20 µL reaction	Volume per 10 µL reaction
PrimeTime One-Step 4X Broad-Range Master Mix (4X)	1X	5 µL	2.5 µL
Forward and reverse primers	250–1000 nM each	Varies	Varies
Probe(s)	150–250 nM each	Varies	Varies
RNA template (do not add yet)	2 pg to 100 ng	2–5 µL	2–4.5 µL
Nuclease-Free Water		Up to 5 µL*	Up to 2.5 µL*

* For purified samples, larger volumes are possible, but will need to be determined empirically.

Direct amplification of specimens in transport media			
Component	Final concentration or amount	Volume per 20 µL reaction	Volume per 10 µL reaction
PrimeTime One-Step 4X Broad-Range Master Mix (4X)	1X	5 µL	2.5 µL
Forward and reverse primers	250–1000 nM each	Varies	Varies
Probe(s)	150–250 nM each	Varies	Varies
Template specimen (Do not add template until the next section)	2 pg to 100 ng	2 µL	1 µL
Direct Amplification Enhancer*	1x	1–2 µL	0.5–1 µL
Nuclease-Free Water		Up to 20 µL	Up to 10 µL

*The Enhancer is generally used for crude specimens. For NP samples, store directly in VTM/UTM. For liquid samples (saliva, other) store at a 1:1 ratio in VTM/UTM. As a guideline, testing with a broad survey of VTM/UTM formulations indicates that 1µL of Direct Amplification Enhancer per 20 µL is appropriate for saliva samples stored in VTM.

3. Dispense equal aliquots of the Reaction Mix into the wells of a qPCR plate that is compatible with your real-time PCR instrument.

Add DNA/RNA template

1. Add direct specimen, extracted RNA template, or controls, to the wells of the qPCR plate that contain Reaction Mix.

Note: At this point, the total volume should be either 20 µL or 10 µL, depending on your chosen reaction volume.

2. Seal the qPCR plate with optically transparent film.
3. Agitate by mixing or briefly vortexing, then centrifuge briefly to remove air bubbles and collect the reaction at the bottom of the wells.

Set up the PCR cycling program

Program the appropriate PCR cycling protocol on your real-time PCR instrument (refer to Table 2).

Table 2. PCR cycling protocol.

Standard RT-qPCR Reaction using purified RNA or direct amplification of specimens in transport media				
Step	Cycles	Temperature (°C)	Standard Cycling (min:sec)	Fast cycling (min:sec)*
Reverse transcription	1	50	15:00	15:00
Polymerase activation	1	95	3:00	0:30
Amplification:				
Denaturation	35–45	95	0:15	0:05
Annealing/extension†		60	1:00	0:45

*** Fast cycling parameters may (in some cases) reduce sensitivity and/or signal.**

† This is a general starting point. The ideal annealing/extension temperature or time may need to be empirically determined.

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

- https://www.idtdna.com/pages/support/safety-data-sheets
- https://www.idtdna.com/pages/support/certificate-of-analysis-search

For additional information or assistance, Contact us.