PrimeTime® Gene Expression Master Mix

Products	Quantity*	Catalog #
PrimeTime® Gene Expression Master Mix	1 x 1 mL	1055770
	1 x 5 mL	1055772
	5 x 5 mL	1055771
	20 x 25 mL	1072102

* Separate tubes of reference dye are included with all orders, except for the 20 x 25 mL size (if needed, reference dye can be ordered by contacting **custcare@idtdna.com**).

Go to **www.idtdna.com/qPCRmastermix** for safety data sheets (SDSs) and certificates of analysis (COAs) for IDT products. Visit **www.idtdna.com/protocols** to verify that you are using the most current version of this protocol.

Contents and storage conditions

PrimeTime® Gene Expression Master Mix is a ready-to-use, 2X concentrated master mix that is designed for use in **probe-based**, real-time quantitative PCR. PrimeTime Gene Expression Master Mix contains an antibody-mediated, hot-start DNA polymerase, dNTPs, $MgCl_2$, enhancers, and stabilizers.

In addition, 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 Gene Expression Master Mix is shipped at ambient temperature. 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
Avoid repeated freezing and thawing
Avoid prolonged exposure of reference dye to light

Prepare reaction mix and run PCR

1. Prepare reagents

a. Thaw the PrimeTime Gene Expression Master Mix, reference dye, primer and probe solutions, and template DNA on ice.

b. Briefly vortex to mix reagents thoroughly.

c. Quickly centrifuge to collect solutions at the bottom of tubes.

2. Add reference dye, as needed

If needed (see Table 1), add the appropriate amount of reference dye to the PrimeTime Gene Expression Master Mix (Table 2).

Table 1. Reference dye concentration levels required by various PCR systems. For instruments not listed, please check with the manufacturer.

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

Table 2. Amount of reference dye to add to PrimeTime® Gene Expression Master Mix.

	Dye volume (µL)	
	High reference dye systems	Low reference dye systems
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

3. Prepare the Reaction Mix

a. Make enough Reaction Mix for the number of reactions needed.

Note: Include controls (no reverse transcriptase, if needed; no template control; positive control) and 1–3 additional reactions to account for pipetting errors.

b. Combine and thoroughly mix all components (Table 3), except for the DNA template, which will be added separately in Step 4.

Note: Add enough Nuclease-Free Water (Catalog # 11-04-02-01 or 11-05-01-14) so that the total reaction volume at the end of Step 4 is 20 µL or 10 µL, depending on your chosen reaction volume.

Table 3. Reaction mixes. The volumes provided are "per reaction". Thus, determine final component volumes by multiplying each volume by the total number of reactions.

PrimeTime® qPCR Assay reactions (Premixed primers and probe)			
Component	**Final conc. or amount**	**Volume per 20 µL reaction**	**Volume per 10 µL reaction**
PrimeTime® Gene Expression Master Mix (2X)	1X	10 µL	5 µL
PrimeTime® qPCR Assay* (20X)	1X	1 µL	0.5 µL
DNA template (Add in Step 4)	3 pg to 100 ng	2–5 µL	2–4.5 µL
Nuclease-Free Water		Bring to 20 µL	Bring 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® Gene Expression 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
DNA template (Add in Step 4)	3 pg to 100 ng	2–5 µL	2–4 µL
Nuclease-Free Water		Bring to 20 µL	Bring to 10 µL

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

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

4. Add DNA template

a. Add 3 pg to 100 ng of DNA template or controls to the wells containing Reaction Mix (Table 3).

b. Seal with optically transparent film.

c. Gently agitate to ensure thorough mixing, and centrifuge briefly to remove air bubbles and collect the reaction at the bottom of the wells.

5. Set up cycling program

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

Table 4. Cycling protocol.

Step	Cycles	Temperature	Fast cycling (min:sec)	Standard cycling (min:sec)
Polymerase activation	1	95°C	3:00	3:00
Amplification:	35–45			
Denaturation		95°C	0:05	0:15
Annealing/Extension*		60°C	0:30	1:00
Hold, if needed	1	4°C	Up to 24 hr	Up to 24 hr

* This is a general starting point. The annealing/extension temperature or time may need to be adjusted based on primer sequences. However, **do not change the polymerase activation conditions**.

6. Run PCR

a. If the plate was stored before PCR, vortex and then centrifuge briefly.

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

For additional information or assistance, visit **www.idtdna.com/protocols** to download the *PrimeTime® qPCR Application Guide* or email us at **applicationsupport@idtdna.com**.

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