Terra™ qPCR Direct TB Green® Premix Protocol-At-A-Glance

Please read the Terra qPCR Direct TB Green Premix User Manual before using this Protocol-At-A-Glance. This abbreviated protocol is provided for your convenience, but it is not intended for first-time users.

1. Prepare your template. Terra qPCR Direct TB Green Premix (Cat. No. 638319) can amplify targets from purified DNA, cell lysates or tissue extracts, and whole cells. You may use any template preparation method that is appropriate for your sample type.

2. Prepare the Master Mix:

    a. Prepare the Master Mix on ice by adding each of the components indicated in Table I.

    b. Make sure you use enough of each reagent for all of your reactions, plus an additional reaction to accommodate pipetting errors.

Table I. Recommended Master Reaction Mixes for Different Real-Tim PCR Instruments.

	qPCR instrument			
	Roche LightCycler	Takara Thermal Cycler Dice™ Real Time System	ABI PRISM 7000 & ABI 7500/7500 Fast Real-Time PCR Systems	
Reagent	**Reagent volume (µl per well) for each instrument**			
Sterile PCR-Grade Water	7.2	9.5	6.8	18.0
Terra PCR Direct TB Green Premix (2X)	10.0	12.5	10.0	25.0
Forward Primer (10 µM)[a]	0.4	0.5	0.4	1.0
Reverse Primer (10 µM)[a]	0.4	0.5	0.4	1.0
ROX Reference Dye LSR or LMP (50X)[b]	–	–	0.4	1.0
Template[c]	2.0	2.0	2.0	4.0
Total volume per well	**20.0**	**25.0**	**20.0[d]**	**50.0[e]**

[a] Use 0.1–1.0 µM of each primer per reaction. For most targets, 0.2 µM of each primer is adequate.

[b] The kit is supplied with two different ROX formulations that allow you to normalize fluorescence signals on instruments that are equipped with this option. ROX Reference Dye LSR is for instruments whose excitation source is a 488-nm laser, while ROX Reference Dye LMP is for instruments whose excitation source is either a lamp or an LED. **Be certain to use the formulation that is appropriate for your real-time instrument!**

[c] The quantity of template required depends on the number of target copies present in the sample. Make serial dilutions to select an appropriate quantity to use. Use no more than 100 ng of template per 20–25 µl reaction. If using single-stranded cDNA as a template, the volume of the cDNA added should be no more than 1/10th the volume of the PCR reaction.

[d] This 20 µl reaction is for ABI instruments using 384-well plates and 96-well Fast Thermal Cycling plates.

[e] This 50 µl reaction is for ABI instruments using 96-well plates, single tubes and 8-tube strips.

3. Mix the Master Mix well by tapping the tube at least 5 times, then centrifuge briefly. Transfer the amount of Master Mix indicated for your real-time instrument (see the last row of Table I) into each well of a PCR plate or 8-well strip, or individual PCR capillary tubes. Centrifuge briefly.

4. Seal the wells according to the procedure recommended for the real-time instrument being used.

5. Program your thermal cycler using the cycling conditions recommended in Table II as a guide (see next page).

Table II. Recommended Thermal Cycling Conditions for Different Real-Time PCR Instruments.

	qPCR instrument							
	Roche LightCycler		Takara Thermal Cycler Dice Real Time System		ABI PRISM 7000 & ABI 7500 Real-Time PCR System		ABI 7500 Fast Real-Time PCR System	
Reaction cycles	**Thermal cycling conditions for each instrument**							
Initial Denaturation[a] (1 cycle):	98°C	2 min[a,b]	98°C	2 min[a]	98°C	2 min[a]	98°C	2 min[a]
qPCR (40 cycles):	98°C 60°C 68°C	10 sec[b] 15 sec[b] 1 min/kb[b,c]	98°C 60°C 68°C	10 sec 15 sec 1 min/kb[c]	98°C 60°C 68°C	10 sec 15 sec 1 min/kb[c]	98°C 60°C 68°C	10 sec 15 sec 1 min/kb[c]
Melting/Dissociation Curve (1 cycle):	95°C 65°C 95°C	0 sec[b] 15 sec[b] 0 sec[b]	98°C 60°C 98°C	15 sec 30 sec 15 sec	Dissociation Curve		Melt Curve	

[a] **The initial denaturation step must be performed at 98°C for 2 min in order to denature the hot-start antibody.**

[b] 20°/sec.

[c] 30 sec for 500 bp or less.

6. Spin the reactions briefly, then place them into the real-time PCR instrument and begin thermal cycling.
7. After the reaction is complete, verify the amplification and melting curves. Establish a standard curve when quantitative analysis is necessary.

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