qPCR Human Reference Total RNA Protocol-At-A-Glance

qPCR Human Reference Total RNA is a mixture of total RNAs from multiple adult human tissues chosen to represent a broad range of expressed genes. It is a reliable reference standard for the accurate and reproducible comparison of gene expression data using real-time quantitative PCR (qPCR). Our qPCR Reference RNA may also be used as a source of positive control templates for validating qPCR primer designs.

This Protocol-at-a-Glance provides a sample qPCR protocol using our TB Green® Advantage® qPCR Premix (Cat. No. 639676) to be used once the individual researcher has generated first-strand reference cDNA with the protocol of their choice.

I. List of Components

- qPCR Human Reference Total RNA (Store at –70°C)

II. Additional Materials Required

For qPCR amplification:

- TB Green Advantage qPCR Premix (Cat. No. 639676)
- qPCR primers
- Supplies and equipment for performing qPCR
- First strand cDNA from an RT reaction, with a minimal concentration of 50 ng/µl input RNA.

III. Protocol: qPCR Amplification

This protocol is a guide to performing qPCR using the first-strand reference cDNA and TB Green Advantage qPCR Premix.

1. If needed, dilute the reaction mixture from the reverse transcriptase reaction down to a final concentration of 10 ng/µl input RNA.
2. Prepare a series of six cDNA reference standards, following the guidelines presented in Table I. Include a no-template control (40 µl RNase-free H_2O) as an additional standard (Standard No. 7).

Table 1. Guidelines for serial dilution

Standard No.	RNA concentration	Aliquot	Volume of RNase-free H_2O to add
1	10 ng/µl	None	None
2	2 ng/µl	10 µl Standard No. 1	40 µl
3	0.4 ng/µl	10 µl Standard No. 2	40 µl
4	0.08 ng/µl	10 µl Standard No. 3	40 µl
5	0.016 ng/µl	10 µl Standard No. 4	40 µl
6	0.0032 ng/µl	10 µl Standard No. 5	40 µl
7	0 ng/µl	N/A	40 µl

3. Prepare a qPCR Master Mix for all reaction wells, plus one additional well. Combine the following components in the order shown:

per rxn	
X µl	RNase-free H_2O
12.5 µl	TB Green Advantage qPCR Premix (2X)
Y µl	Primer mix (20–400 nM)*
20 µl	Total volume

* The amount varies according to application.

4. Mix well by vortexing and spin the tube briefly in a microcentrifuge.
5. Aliquot 20 µl of qPCR Master Mix into each well of a 96-well optical PCR plate.
6. Add 5 µl of Standard No. 1 (from Step 1, Table I) to a well. Repeat, adding the next Standard in the series to each subsequent well, to obtain a qPCR reaction for each Standard. Seal the plate using optical strip caps.
7. Begin thermal cycling using the parameters optimized for your primers.

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