

# Human Universal Reference Total cDNA

A high-performance standard for quantitative PCR

- **Broad gene coverage**
- **Virtually free of genomic DNA**
- **Made from human tissues, not cultured cell lines**

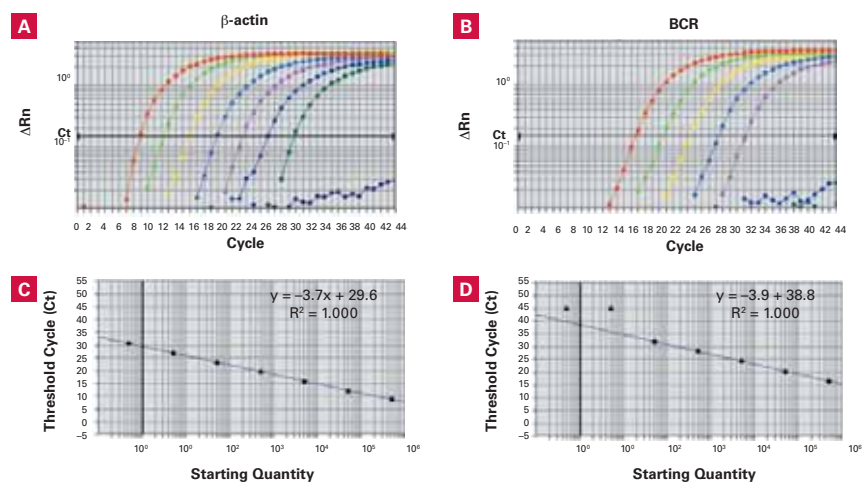
Measurements of mRNA expression levels—whether by Northern blotting, ribonuclease protection, or real-time quantitative PCR—are usually standardized by comparing the data to that obtained for an internal or endogenous reference gene. Housekeeping genes such as  $\beta$ -actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are most often used because their expression levels are expected to remain constant under different treatment conditions. Unfortunately, this assumption is not always valid, and results based on housekeeping genes alone can be biased (1). A better method is to normalize your data using our **Human Universal Reference Total cDNA\***, the only total cDNA control derived entirely from human tissues (2).

Human Universal Reference Total cDNA is the ideal control for comparing data from different quantitative PCR (qPCR) experiments. Because it is prepared from a total RNA pool collected from several different tissues, Human Universal Reference cDNA provides broad gene coverage, as shown by microarray analysis of the RNA starting material (Figure 1). In fact, RNA, and therefore cDNA, prepared from whole tissues provides better gene representation with less variation than RNA made from cell lines (Figure 1). What's more, PCR analysis shows that our Total RNA is virtually free of genomic DNA (3). This allows for a more accurate measurement of transcript copy number. And both high and low abundance genes are well represented allowing you to prepare a wide range of serially diluted standards for each qPCR assay (Figure 2).

The lot-to-lot variation of our Universal cDNA is minimal because the RNA source is prepared on an industrial scale. For accurate, reliable results use Universal



**Figure 1. Hybridization of Human Universal Reference RNA to the Atlas® Glass Human 3.8 I Microarray.** We generated Cy-3 labeled probes using our Reference Total RNA and RNA from single tissues and hybridized the probes to Atlas Glass Human 3.8 I Microarrays (Cat. No. 634638). We analyzed the expression results using GeneSpring software, which clusters genes according to their expression patterns. Among the genes detected with the Reference Total RNA, 83% had intensities greater than or equal to the intensity obtained with the hybridization of single tissue RNA samples. Nearly all of the genes expressed in individual tissues were detected using our Reference Total RNA. These data indicate that our Reference Total RNA has more than 92% gene coverage and outperforms a competitor's reference RNA mixture, prepared from cell lines. The black and varying shades of gray indicate high and low expression, respectively.



**Figure 2. High, medium, and low abundance genes are well represented.** 10-fold serial dilutions of our Human Universal Reference Total cDNA were analyzed by quantitative PCR using Q-Zyme™ GS (Gene Specific) Assays (coming soon) for  $\beta$ -actin, a high abundance gene (Panel A), and BCR, a low abundance gene (Panel B). The corresponding standard curves are shown in Panels C & D. All seven dilutions contained detectable quantities of  $\beta$ -actin transcripts; the first five contained detectable quantities of BCR transcripts.

Reference Total cDNA. It consistently outperforms other controls.

#### References

1. Suzuki, T. *et al.* (2000) *Biotechniques* 29:332–337.
2. Control RNA for microarray experiments (April 2002) *Clontechiques* XVII(2):6.
3. Premium Total RNA contains virtually no genomic DNA, an important factor in RNA quality. (October 2002) *Clontechiques* XVII(4):8–9.

\* We also provide Human Universal Reference Total RNA for use in one-step RT-PCR, Northern blotting, and microarray experiments.

Product	Size	Cat. No.
Human Universal Reference Total cDNA	25 x 50- $\mu$ l rxns	639653
	100 x 50- $\mu$ l rxns	639654
	Human Universal Reference Total RNA	2 x 200 $\mu$ g

# Human Universal Reference Total cDNA...continued

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