BD QZyme™ Assays for Quantitative PCR

Measure the expression level of any human gene

- **Highly sensitive—detect fewer than 10 copies of a DNA target**
- **Easy multiplex analysis—quantify two or more genes in a single tube**
- **Accurate over a broad range of target concentrations—detect differences of up to 5 orders of magnitude**
- **Bright fluorescent signal—works on any qPCR instrument**

Introducing the **BD QZyme™ Assay** for quantitative PCR (qPCR), a novel DNA amplification system for the real-time detection and quantification of specific cDNA and genomic DNA targets. Compatible with all real-time PCR instruments and readily adapted for use in single or multiplex analyses, BD QZyme Assays can accurately measure fewer than 10 copies of target DNA. The assays are easy to set up and require no optimization since they rely on a single set of PCR cycling parameters, which can be universally applied for the detection of any genomic DNA or mRNA target. The dynamic range, or ability of the assay to accurately measure differences in target concentration, is extraordinarily broad, typically extending over 5 orders of magnitude for high-abundance genes.

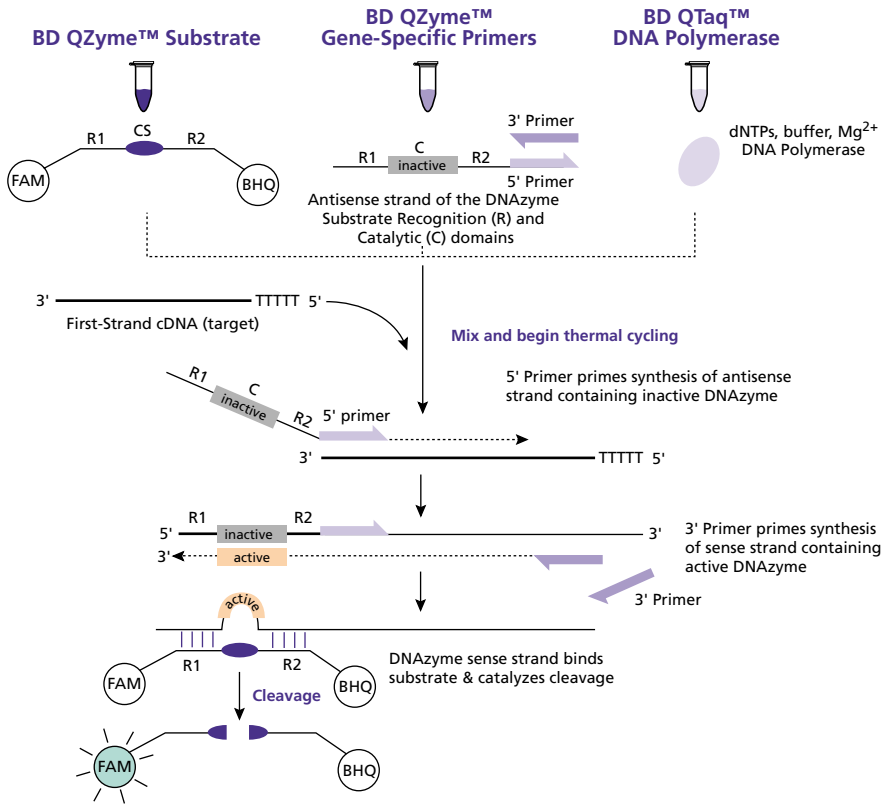

Figure 1. The BD QZyme™ Assay. The 5' Primer is comprised of a target-specific sequence joined to the inactive (antisense) strand of the DNAzyme. During amplification, amplicons are produced that contain active (sense) copies of the DNAzyme. The accumulation of amplicons is accompanied by an increase in fluorescence, produced by the action of the DNAzyme on its fluorogenic substrate. Elements not drawn to scale. CS = Cleavage Site.

The BD QZyme™ Assay

The principle of the BD QZyme Assay is similar to that of other quantitative PCR systems, but the mechanism is quite different. The central component is the patented DNAzyme, a catalytically active oligonucleotide that cleaves nucleic acid substrates at specific phosphodiester bonds. The DNAzyme consists of a catalytic domain (C) flanked by two substrate recognition domains (R1 & R2). It observes standard Watson-Crick base-pairing rules and must hybridize with its substrate to properly catalyze the cleavage. This activity has been harnessed to develop the real-time, quantitative PCR assay shown in Figure 1. The essential reagents include a gene-specific 5' primer conjoined to the inactive, antisense strand of the DNAzyme; and a gene-specific 3' primer. Also present is a DNAzyme-specific fluorogenic substrate, a short nucleic acid segment tagged with

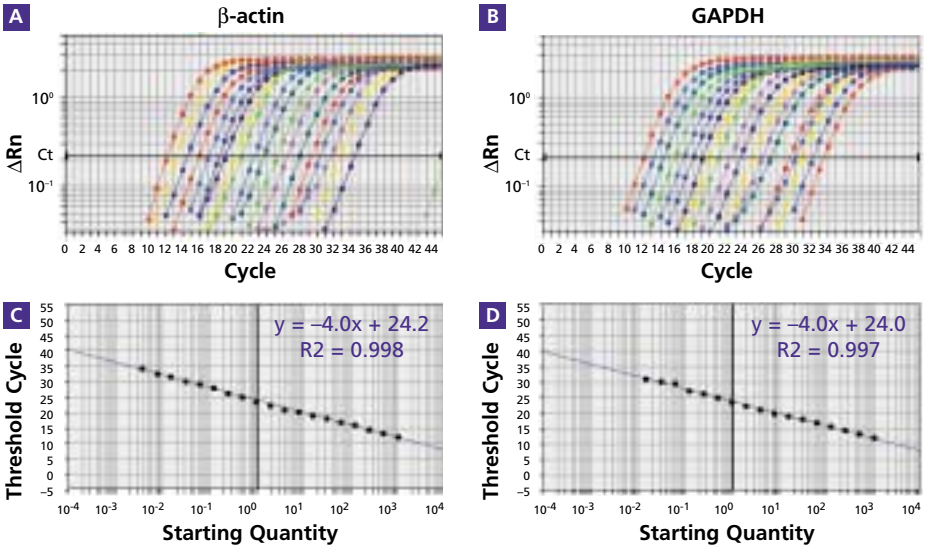

Figure 2. The dynamic range of the BD QZyme™ Assay extends over 5 orders of magnitude. Serial 2-fold dilutions of Human Universal Reference Total cDNA (Cat. No. 636692) were analyzed by real-time PCR on an ABI Prism 7700 using the BD QZyme Assays for β-actin (**Panel A**) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; **Panel B**). Each curve represents a different dilution. The standard curves (C_t value vs. starting quantity of cDNA) for each assay are shown in **Panels C** and **D**.

BD QZyme™ Assays...continued

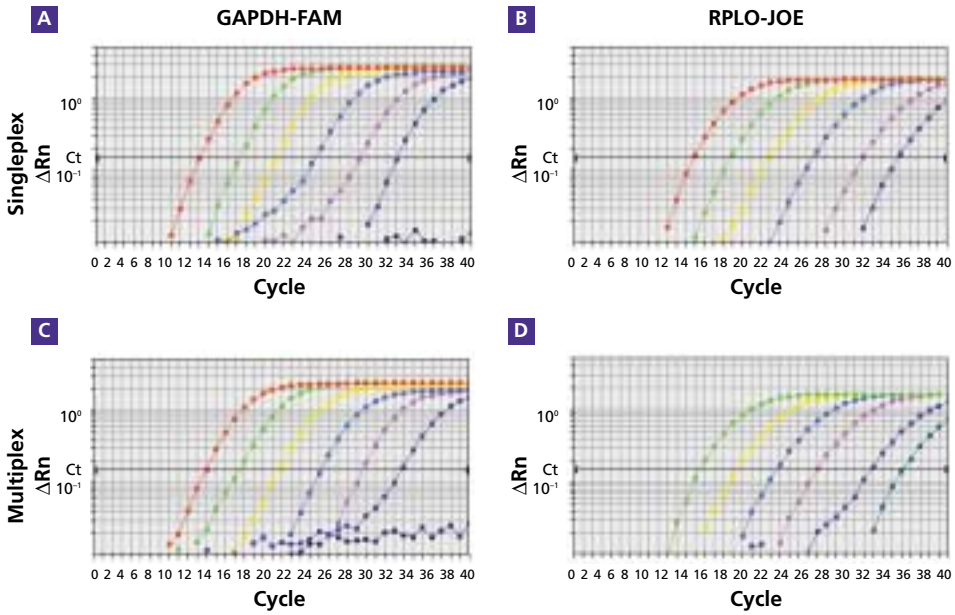

Figure 3. The BD QZyme™ Assay is easily adapted for multiplex analysis. Using different DNAzyme-substrate pairs, which we provide, you can quantify two genes simultaneously, in one reaction. As an example, serial 10-fold dilutions of Human Universal Reference Total cDNA (Cat. No. 636692) were analyzed by real-time PCR using the BD QZyme Assays for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and ribosomal protein L15 (RPLO). The genes were analyzed separately (**Panels A & B**) and simultaneously (**Panels C & D**), using spectrally distinct DNAzyme substrates: A FAM-labeled substrate to measure GAPDH amplification (**Panels A & C**), and a JOE-labeled substrate to measure RPLO amplification (**Panels B & D**). Note that the amplification curves for the corresponding singleplex and multiplex analyses are similar, as expected from the specificity of the BD QZyme Assay.

a fluorophore at one end and a Black Hole Quencher™ dye (BHQ) at the other.

The DNAzyme substrate ultimately serves as a measure of DNA copy number. With each round of amplification, the number of active DNAzymes increases in direct proportion to the DNA copy number. The exponential increase in DNAzyme activity leads to a corresponding increase in fluorescence, allowing you to monitor DNA amplification in real time. As in other real-time PCR systems, the more copies of target you start with, the fewer cycles needed to reach a threshold level of detection—the C_t value. Background fluorescence is extremely low. The BD QZyme fluorophores, which include FAM, TET, and JOE, represent some of the brightest available, emitting strongly in their excited states but only weakly in their quenched states. In fact, the signals produced by these fluorophores are equal to—or greater than—those of the leading qPCR chemistries.

Universal protocol—no optimization required

The BD QZyme Assay has a number of important advantages over other quantitative PCR systems. First, it requires no optimization; the reaction set up and thermal cycling parameters are the same regardless of the gene you wish to analyze. Second, the assay is platform independent; you can prepare your samples in 96-well or 384-well plates, and analyze the reactions using any commercially available real-time PCR instrument, including the ABI Prism 7000, 7700, or 7900; Roche LightCycler; or Bio-Rad iCycler. And third, BD QZyme Assays are unaffected by the complexity of the cDNA sample. The sample can be a simple solution consisting of a single cDNA species, prepared by one-step or two-step RT-PCR, or a complex mixture comprising an entire cDNA or genomic DNA library.

Product	Size	Cat. No.
BD QZyme Assay❖	200 x 50 µl Assays	many

NEW!

❖ Coming soon. Please inquire about availability.

Components

- 100X BD QZyme™ Gene-Specific Primers (5' & 3')
- 100X BD QZyme™ Substrate

Related Products

- BD™ Human Universal Reference cDNA (Cat. Nos. 636693, 636692, 639654, 639653)
- BD QTaq™ DNA Polymerase Mix (Cat. Nos. 639651, 639652, 639655).
- Matched Tumor/Normal cDNA Pairs (many)
- BD™ Premium RNA (many)

Notice to Purchaser

Please see the PCR and BD QZyme™ Products legal statements on page 19.

Both practical and dependable, the BD QZyme Assay can accurately measure DNA copy number over an extremely broad range, from as few as three copies to as many as 10^6 copies or more—a linear dynamic range extending over 5 logs (Figure 2). The assay is easily adapted for multiplex analysis by including spectrally distinct BD QZyme substrates (Figure 3). Gene specificity is maintained by using different DNAzymes, each specifically designed to recognize and cut a single substrate.

Pretested for optimal results

Each BD QZyme Assay includes a premixed solution of gene-specific primers (5' and 3') along with a stock solution of DNAzyme substrate—sufficient for 200 50-µl singleplex assays. DNA polymerase and dNTP mix must be obtained separately. For best results, we recommend BD QTaq™ DNA Polymerase Mix (see pages 4–5), which has been optimized for use with BD QZyme™ Assays. Every BD QZyme Assay is pretested with our Human Universal Reference Total cDNA—the only cDNA standard made from human tissues—for guaranteed performance over a defined template concentration range.