SYBR® Green Cells-to-CT™ Kits

Simple, Complete Workflows for Gene Expression Analysis without RNA Purification

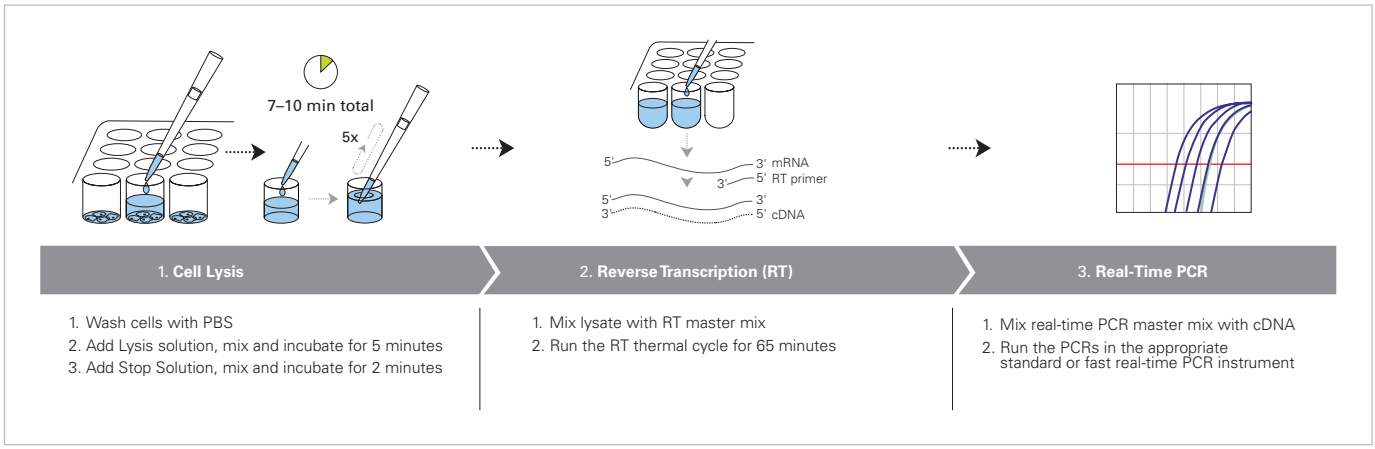

Figure 1. SYBR® Green Cells-to-CT™ Kits Ready for RT in Just 7 Minutes. The SYBR Green Cells-to-CT Kits require only 7 minutes at room temperature to release nucleic acids into a cell lysate solution that is compatible with the included reverse transcriptase and real-time PCR reagents.

- **Extraordinary Value**—Complete kit format includes pre-optimized reagents to work efficiently and robustly right out of the box; includes cell lysis reagents, DNase, reverse transcription (RT) reagents, and *Power* SYBR® Green or Fast SYBR® Green Master Mix
- **Extraordinary Ease**—Simple, effective Cells-to-CT™ methodology enables sample preparation at room temperature in only 7 minutes, including DNase treatment
- **Extraordinary Performance**—Validated accuracy, exceptional reproducibility, and maximal sensitivity from 10 to 100,000 cells per sample; results equivalent to those from purified RNA

Extraordinary Value

Power SYBR Green and Fast SYBR Green Cells-to-CT Kits take you from cultured cells to real-time PCR results with the fastest, easiest, and most robust workflow available today. A breakthrough cell lysis and RNA stabilization technology completely eliminates the need for laborious and time consuming RNA purification. However, the SYBR Green Cells-to-CT Kits don't stop at sample preparation. They integrate the lysis technology into a complete, optimized gene expression workflow, that includes reverse transcription reagents and high performance *Power* SYBR Green or Fast SYBR Green Master Mixes. All kit components have been validated on primer sets targeting hundreds of genes. The trial and error associated with the use of separate sample preparation, RT, and real-time PCR kits has been removed, enabling successful results for novice or experienced researchers, right out of the box. The *Power* SYBR Green and Fast SYBR Green Cells-to-CT Kits offer extraordinary simplicity, performance, and value over traditional SYBR Green-based gene expression analysis workflows, as well as competitor lysate-based kits.

Extraordinary Ease

The SYBR Green Cells-to-CT protocol begins with a simple 7-minute sample preparation procedure illustrated in Figure 1. Starting with 10 to 100,000 cultured cells/sample, cells are washed in PBS, and then lysed for 5 minutes at room temperature; DNase treatment can be performed concurrently. Lysis is terminated at room temperature by

adding Stop Solution and incubating for 2 minutes. The lysates are then ready for reverse transcription or storage at –20°C for up to 5 months. Unlike old-fashioned multi-step RNA isolation protocols, *Power* SYBR® Green and Fast SYBR® Green Cells-to-CT™ Kits do not require heating, washing, or centrifugation steps thus streamlining a laborious, repetitive pipetting process to a mere 2-step, 7-minute procedure. Because samples can be processed directly in culture plates (96- or 384-well), sample handling and the potential for sample loss or transfer error are minimized, resulting in higher reproducibility.

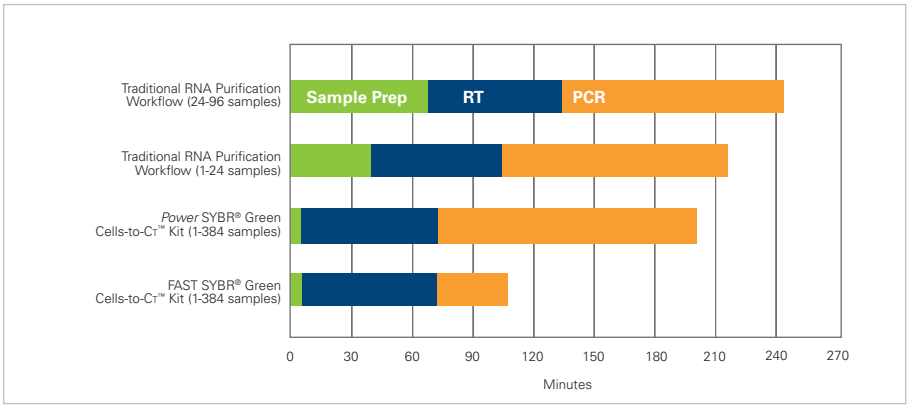

Figure 2. SYBR® Green Cells-to-CT™ Kits Decrease Time to Results. The time required to complete gene expression analysis experiments from cultured cells using SYBR Green Cells-to-CT Kits is up to 60% less than that required for traditional RNA purification workflows. Additionally, the Cells-to-CT Kits eliminate repetitive pipetting steps commonly used in traditional RNA purification methods, and can easily be scaled for processing a small number of samples in single tubes, or up to 384-well sample plates.

Following sample preparation, a portion of the cell lysate is added to an RT reaction, and real-time PCR performed using either *Power* SYBR Green or Fast SYBR Green Master Mix. All the necessary reagents are included in the SYBR Green Cells-to-CT Kits (excluding user-specified PCR primer sets specific to your targets of interest).

As illustrated in Figure 2, *Power* SYBR Green and Fast SYBR Green Cells-to-CT workflows offer considerable time savings compared to workflows utilizing traditional RNA purification methods. In addition, the Cells-to-CT Kits can easily be scaled for processing single tubes or up to 384-well sample plates. In contrast, traditional purification methods can be difficult to scale up manually, often requiring expensive centrifuge or vacuum platforms.

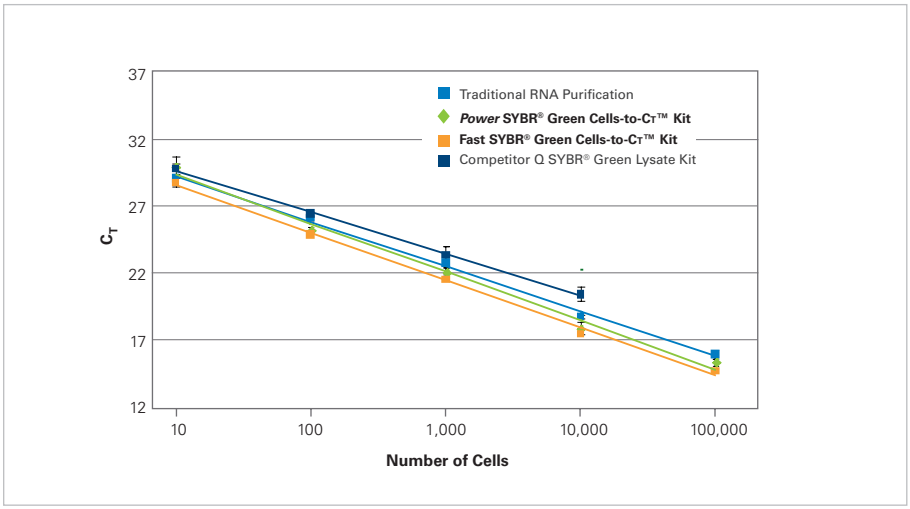

Figure 3. *Power* SYBR® Green and Fast SYBR® Green Cells-to-CT™ Kits Perform Equivalently to Purified RNA and are Superior to Competitor Lysate Kits. HeLa cells (10 to 10^5) were prepared using each manufacturer's recommended protocol with: 1) traditional glass fiber filter RNA purification and real-time RT-PCR using the Applied Biosystems High Capacity RNA-to-cDNA Kit and *Power* SYBR Green PCR Master Mix (slope = -3.3, R^2 = 0.99); 2) *Power* SYBR Green Cells-to-CT Kit (slope = -3.6, R^2 = 0.99); 3) Fast SYBR Green Cells-to-CT Kit (slope = -3.5, R^2 = 0.99); or 4) Competitor Q SYBR Green lysate kit (slope = -3.1, R^2= 0.99). Real-time PCR was performed using a primer set targeting ß-actin.

Extraordinary Performance

Dynamic Range, Sensitivity, and Reproducibility Compared to Purified RNA

To demonstrate the performance of the SYBR Green Cells-to-CT Kits, replicate experiments were run using either traditional RNA purification and real-time PCR analysis, or the *Power* SYBR Green and Fast SYBR Green Cells-to-CT Kits. Both kits show equivalent performance to results obtained using purified RNA for analysis of 10 to 100,000 cells (Figure 3). In addition, the sensitivity, efficiency, and dynamic range of both SYBR Green Cells-to-CT Kits were superior to competitor lysate kits analyzed (Figure 3). The observed dynamic range using Competitor Q lysate kit was 10 to 10^4 cell equivalents, or 1 log less than the *Power* SYBR Green and Fast SYBR Green Cells-to-CT Kits.

The high level of sensitivity of both SYBR Green Cells-to-CT Kits can be attributed to several key features. First, the ability to incorporate large sample volumes in the RT and real-time PCR reactions result in maximum sample input. Up to 45% of the total RT reaction volume can be cell lysate, and up to 30% of the real-time PCR volume can be cDNA.

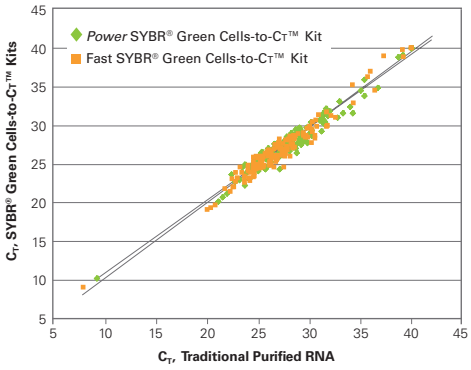

Figure 4. *Power* SYBR® Green and Fast SYBR® Green Cells-to-CT™ Kits Perform Equivalently to Purified RNA Over a Broad Set of Gene Targets. A cell mixture (10,000 cells) comprised of HeLa, HepG2, Jurkat, HEK-293, and U-87-MG cells were analyzed using either traditional RNA purification and real-time RT-PCR, or the *Power* SYBR Green and Fast SYBR Green Cells-to-CT Kits. Technical (real-time PCR) quadruplicates were performed for each method for each of the 155 primer sets. The average C_T values for the technical replicates are shown. A high correlation was observed between the traditional purified RNA real-time RT-PCR results to the *Power* SYBR Green Cells-to-CT Kit (slope = 0.94, R^2 =0.96) and Fast SYBR Green Cells-to-CT Kit (slope = 0.97, R^2 = 0.96).

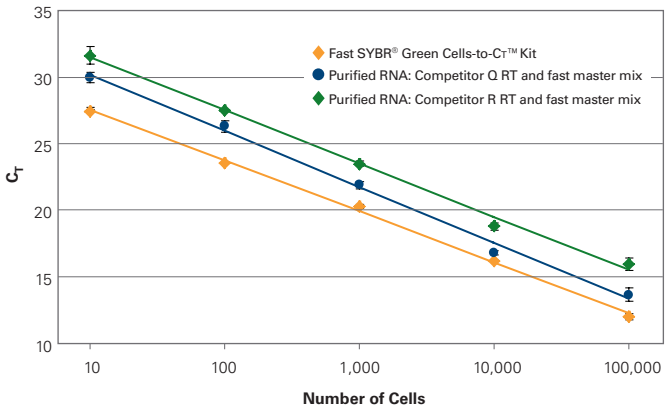

Figure 5. Fast SYBR® Green Cells-to-CT™ Kit Performance is Superior to Competitor Real-Time PCR Reagents using Purified RNA. RNA from a 10-fold serial dilution of HeLa cells (10 to 100,000 cells) was analyzed using the Fast SYBR Green Cells-to-CT Kit (slope = -3.8, R^2 = 0.99), or workflows using purified RNA with RT and fast master mixes from Competitor Q (slope = -4.2, R^2 = 0.99) or Competitor R (slope = -4.0, R^2 = 0.99). Experiments were run using a ß-actin primer set. All experiments were performed according to each manufacturer's maximum recommended volumes.

Next, sample handling is minimized, resulting in highly efficient retention of target molecules during sample preparation. Finally, the inherent performance characteristics of both *Power* SYBR® Green and Fast SYBR® Green Master Mixes allow for sensitive, specific, and dependable target quantitation over a wide dynamic range.

SYBR® Green Cells-to-CT™ Kits allow for subtle gene expression changes to be detected with confidence due to high technical reproducibility. Imparted by robust reagents and minimal sample handling in the Cells-to-CT Kit workflow, this exceptional reproducibility is especially beneficial when working with low cell numbers where fluctuation in isolation efficiency or sample loss can have dramatic impact.

High Correlation of Real-Time PCR Results to Purified RNA

The robustness of both SYBR Green Cells-to-CT Kits was analyzed with 155 primer sets to targets with diverse gene expression levels, and compared to results obtained using purified RNA. A high degree of concordance was obtained, spanning a wide range of C_T values, between data generated with purified RNA and that generated with both the *Power* SYBR Green Cells-to-CT Kit and Fast SYBR Cells-to-CT Kit (over 600 data points, Figure 4). The data indicate that the SYBR Green Cells-to-CT Kits can be used for accurate gene expression analysis, with results equivalent to those obtained using purified RNA.

Performance Compared to Competitor Master Mixes

The *Power* SYBR Green PCR Master Mix and Fast SYBR Green Master Mix, included in the respective Cells-to-CT Kits, have been designed for highly specific yet sensitive nucleic acid quantitation over a broad dynamic range. The convenient 2X Master Mixes are formulated for increased sensitivity using SYBR Green I dye, dNTPs, uracil-DNA glycosylase (to reduce carryover contamination), and our proprietary ROX™ passive internal reference dye for increased precision. The *Power* SYBR Green Cells-to-CT Kit is *Power*ed by AmpliTaq Gold® DNA Polymerase, LD which is formulated to provide the highest levels of specificity with standard real-time PCR. The Fast SYBR Green Cells-to-CT Kit contains AmpliTaq® Fast DNA Polymerase, UP, which is designed to allow instant hot start, thus minimizing non-specific product formation with fast real-time PCR.

As seen in Figure 5, Fast SYBR Green Cells-to-CT Kit demonstrated superior performance compared to purified RNA which was reverse transcribed and PCR amplified with Competitor R's or Competitor Q's RT and fast master mix, respectively. Similarly, the *Power* SYBR®

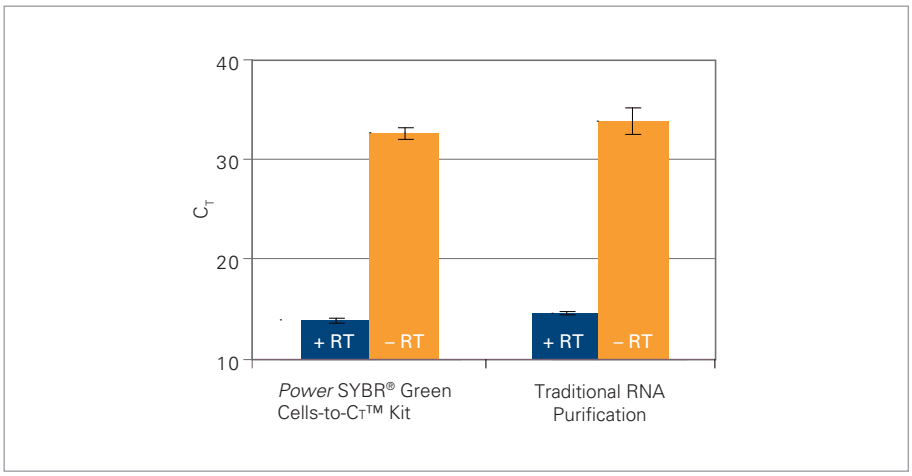

Figure 6. Efficient SYBR® Green Cells-to-Ct™ Kit DNase Treatment Eliminates Contaminating DNA. Eight replicates of 100,000 HeLa cells were prepared using either the *Power* SYBR® Green Cells-to-Ct™ Kit or traditional glass fiber filter purification. Real-time PCR was performed for RNase P (designed to detect a single copy DNA locus) for both reverse transcribed (+RT) and non-reverse transcribed (-RT) RNA samples. Similar ΔC_T (difference between +RT and - RT) results were observed for both the purified RNA and *Power* SYBR Green Cells-to-Ct Kit workflows (ΔC_T = 19.1 vs. 18.7). Similar results were observed for the Fast SYBR® Green Cells-to-Ct Kit and for cell inputs across the dynamic range of the kits (data not shown).

Green Cells-to-Ct™ Kit displayed superior performance compared to competitor SYBR Green I- based master mixes (data not shown). Both *Power* SYBR® Green PCR Master Mix and Fast SYBR® Green Master Mix are formulated to minimize non-specific amplification that could reduce amplification efficiency and accuracy, while delivering maximum sensitivity, reproducibility, and wide dynamic range.

Efficient Removal of Contaminating DNA

Valid real-time PCR data is predicated on amplification of a single intended target representing RNA expression levels. Contaminating genomic DNA (gDNA), non-specific amplification of primer-dimers, and promiscuous priming of homologous sequences can affect data accuracy. The efficiency of the Cells-to-Ct Kits DNase treatment (performed during the 5-minute lysis step) was evaluated by detecting residual gDNA in lysates prepared with the *Power* SYBR Green Cells-to-Ct Kit, and compared to RNA purified with a standard glass fiber filter method. RNA prepared from 100,000 cells served as input for both standard RT reactions (+RT) and non-reverse transcribed controls (-RT), and real-time PCR was performed to detect gDNA contamination. Even at the maximal cell concentration, only negligible gDNA remained in the samples prepared by both methods (<0.0003% of the amplifiable template in the +RT real-time PCRs is gDNA) (Figure 6). This represents removal of approximately 99.6% of the gDNA in the original sample. In addition, extensive validation by melt curve analysis has shown that SYBR Green Cells-to-Ct Kits do not increase non-specific amplification or the incidence of primer-dimer formation, compared to purified RNA samples.

Compatible with a Wide Range of Real-Time PCR Platforms

Applied Biosystems offers industry-leading real-time PCR instrument platforms to meet the needs of laboratories worldwide. However, in labs where legacy non-Applied Biosystems instruments are used, performance of *Power* SYBR Green and Fast SYBR Green Cells-to-Ct Kits are not compromised. Compatibility of the SYBR Green Cells-to-Ct Kits was tested with replicate reactions targeting ß-actin from cDNA generated from both *Power* SYBR Green and Fast SYBR Green Cells-to-Ct Kits and real-time PCR run on Applied Biosystems, Roche, or Bio-Rad real-time PCR platforms. Replicates showed excellent uniformity across all real-time PCR instruments tested (Figure 7). Dissociation curves show a single peak, indicating that the ß-actin target is specifically amplified, thus demonstrating the robustness of the SYBR Green Cells-to-Ct Kits.

Regardless of whether you are using *Power* SYBR Green or Fast SYBR Green Master Mix, or the new SYBR Green Cells-to-Ct Kit workflows, Applied Biosystems is committed to providing the highest quality reagents for all of your real-time PCR needs. For more information on the family of Cells-to-Ct Kits, visit **www.appliedbiosystems.com/c2ct.**

SYBR® Green Cells-to-Ct™ Control Kit

The Cells-to-Ct Kits have been successfully utilized for multiple applications on numerous cell lines including adherent immortalized cells, suspension cell lines, stem cells, as well as primary cells (for a complete list of evaluated cell lines, please visit **www.appliedbiosystems.com/c2ct**). To validate optimal performance with SYBR Green Cells-to-Ct Kits, the SYBR Green Cells-to-Ct Control Kit can be used to monitor efficiency of cell lysis and amplification inhibition using a supplied XenoRNA™ control and primer set. In addition, primers for an endogenous gene target can be used as a positive control to ensure adequate sample input, or for use in relative quantitation.

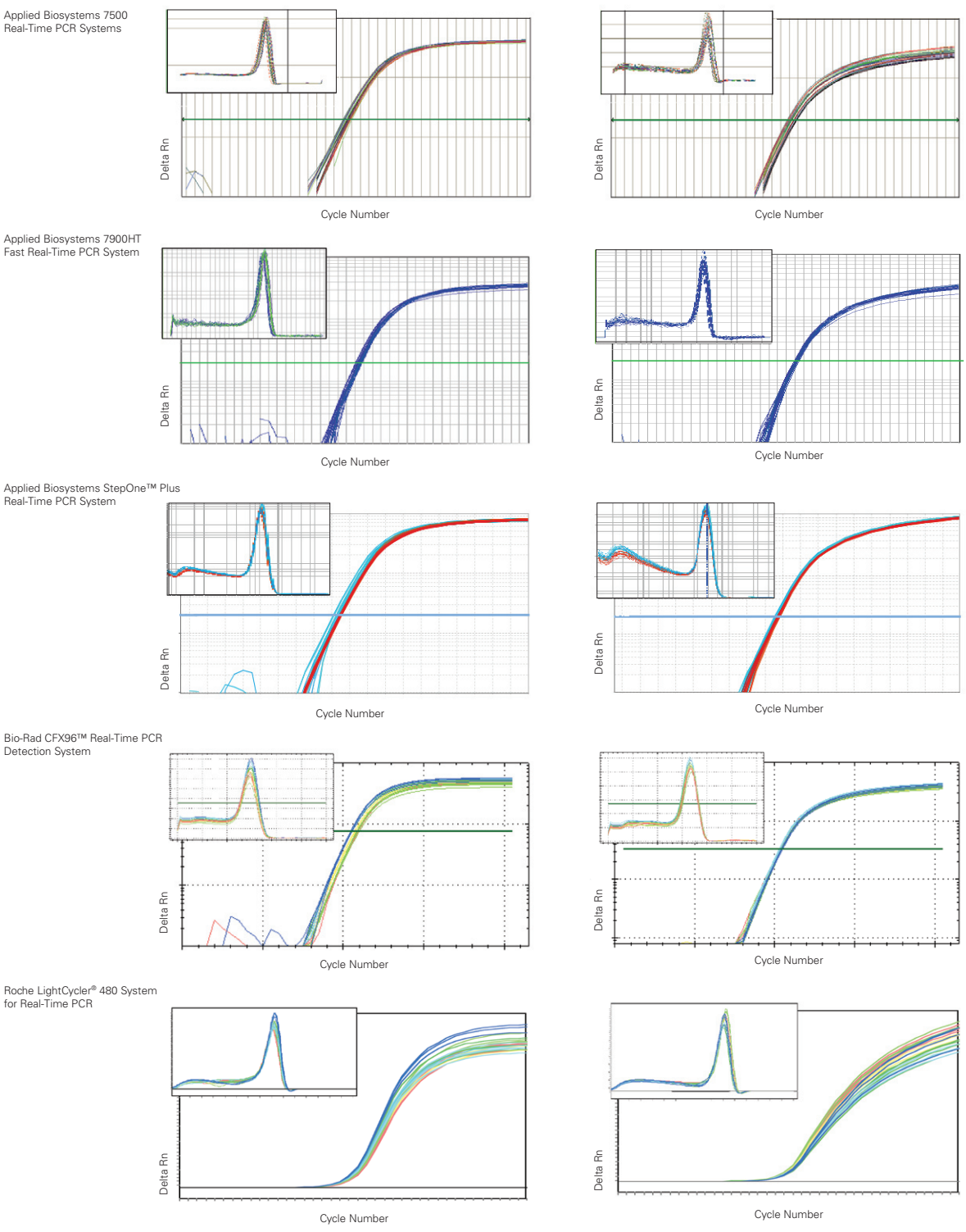

Figure 7. SYBR® Green Cells-to-CT™ Kits are Compatible with Diverse Real-Time PCR Instruments. A cell mixture of HeLa, HepG2, Jurkat, HEK-293, and U-87-MG cells (10,000 cells) was analyzed using either the *Power* SYBR® Green or Fast SYBR® Green Cells-to-CT Kit, or RNA purified by a traditional glass fiber filter method. Replicates were analyzed using a ß-actin primer set following *Power* SYBR Green or Fast SYBR Green Cells-to-CT Kit recommended conditions.

ORDERING INFORMATION

Description	Size	P/N
Power SYBR® Green Cells-to-Ct™ Kit 40 lysis reactions with gDNA removal 40 cDNA synthesis reactions (50 µL) 200 PCRs (20 µL)	40 rxns	4402953
Power SYBR® Green Cells-to-Ct™ Kit 100 lysis reactions with gDNA removal 100 cDNA synthesis reactions (50 µL) 500 PCRs (20 µL)	100 rxns	4402954
Power SYBR® Green Cells-to-Ct™ Kit 400 lysis reactions with gDNA removal 400 cDNA synthesis reactions (50 µL) 2,000 PCRs (20 µL)	400 rxns	4402955
Fast SYBR® Green Cells-to-Ct™ Kit 100 lysis reactions with gDNA removal 100 cDNA synthesis reactions (50 µL) 500 PCRs (20 µL)	100 rxns	4402956
Fast SYBR® Green Cells-to-Ct™ Kit 400 lysis reactions with gDNA removal 400 cDNA synthesis reactions (50 µL) 2,000 PCRs (20 µL)	400 rxns	4402957
SYBR® Green Cells-to-Ct™ Control Kit	100 rxns	4402959

RELATED PRODUCTS

Instruments

StepOne™ Real-Time PCR System		4376357
StepOnePlus™ Real-Time PCR System		4376600
Applied Biosystems 7500 Real-Time PCR System		4351104
Applied Biosystems 7500 Fast Real-Time PCR System		4351106
Applied Biosystems 7900HT Fast Real-Time PCR System,		
with Standard 96-Well Block Module		4329003
with Fast 96-Well Block Module		4351405
with 384-Well Block Module		4329001

Master Mixes

Power SYBR® Green PCR Master Mix	1 mL	4368577
	5 mL	4367659
	2 × 5 mL	4368706
Fast SYBR® Green Master Mix	1 mL	4385610
	5 mL	4385612
	2 × 5 mL	4385616

Reagents

Nuclease-free Water (not DEPC-treated)		AM9938
RNase*Zap*™ Solution		AM9780

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