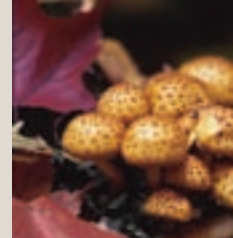


Streamlined Expression Analysis of Multiple Genes Using Cell Lysates with TaqMan® Arrays



TaqMan® Gene Expression Cells-to-Ct™ Kit TaqMan® Arrays

The combination of TaqMan® Gene Expression Cells-to-Ct™ Kit for sample preparation with TaqMan Arrays (micro fluidic cards pre-loaded with up to 384 real-time RT-PCR assays) provides researchers with a fast and accurate workflow for gene expression analysis from cultured mammalian cells. As shown here, when gene expression is assessed with TaqMan Arrays, cell lysates from the TaqMan Gene Expression Cells-to-Ct Kit performed equivalently to purified RNA for most samples and often showed improved sensitivity for samples with very low cell numbers.

Gene Expression Analysis Without RNA Isolation

The TaqMan Gene Expression Cells-to-Ct Kit makes it possible to perform expression analysis directly from cultured cells without RNA purification. This innovative sample preparation procedure features a unique method for lysing cultured cells while removing genomic DNA and preserving RNA integrity. Additionally, the TaqMan Gene Expression Cells-to-Ct Kit contains reverse transcription (RT) reagents for cDNA synthesis, and TaqMan Gene Expression Master Mix for real-time PCR analysis. These components allow users to test their samples directly with TaqMan Gene Expression Assays and TaqMan Arrays (see sidebar, page 29, *TaqMan Arrays for Easy and Robust Gene Expression Analysis*).

In this study, samples were prepared with the TaqMan Gene Expression Cells-to-Ct Kit and an RNA purification kit. After reverse transcription, the samples were loaded on TaqMan Arrays (TaqMan Endogenous Control Array), and the results from cell lysates versus purified RNA were compared.

Sample Preparation

Sample Set 1—Cell Lysates Using the TaqMan Gene Expression Cells-to-Ct Kit. HeLa cells (10–100,000 cells) were washed with PBS and lysed for 5 minutes at room temperature; DNase treatment was performed simultaneously (Figure 1). Lysis was terminated at room temperature by a 2-minute incubation with Stop Solution.

Lysates may be frozen for up to 5 months or can be added directly to the RT reaction. Because samples can be processed directly in culture wells (96 or 384 wells), sample handling and the potential for sample loss or transfer error are minimized, facilitating rapid, high throughput processing. Unlike multi-step RNA isolation protocols, no heating, washing, or centrifugation steps are required. The kit greatly simplifies a 30–60 minute process to 7 minutes.

Sample Set 2—RNA Purification Using Column-Based Protocol. Total RNA was purified from HeLa cells (10–100,000 cells) using a commercially available, column-based kit.

Reverse Transcription (RT). After sample preparation, lysates or purified RNA were combined with the 2X RT Buffer and 20X RT Enzyme Mix for RT. In these experiments (Figures 2–3), sample comprised 20% of the RT reaction volume; however, up to 45% of the RT reaction can be composed of lysate, maximizing sensitivity and sample carry-through.

TaqMan Arrays for Gene Expression Analysis

Master Mix. Using the TaqMan Gene Expression Master Mix included in the TaqMan Gene Expression Cells-to-Ct Kit, cDNA can comprise up to 45% of the real-time PCR volume. This master mix amplifies the target precisely and accurately, enabling the detection of small quantities of template, such as transcripts expressed at low levels.

Real-Time PCR. After RT of cell lysates and purified RNA, the resulting cDNA samples were combined with TaqMan Gene Expression Master Mix and loaded onto TaqMan Human Endogenous Control Arrays. RT products comprised 20% of the PCR volume for each sample. The Endogenous Control Arrays contain primers/probes for 16 commonly used housekeeping genes and genes that exhibit minimal differential expression across a large number of tissues. The arrays were run using universal thermal cycling conditions on a 7900HT Fast Real-Time PCR System.

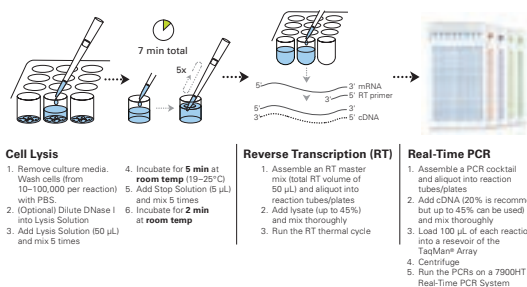


Figure 1. A Simplified Workflow for Analyzing Gene Expression Using TaqMan® Gene Expression Cells-to-Ct™ Kit Lysates and TaqMan® Arrays.

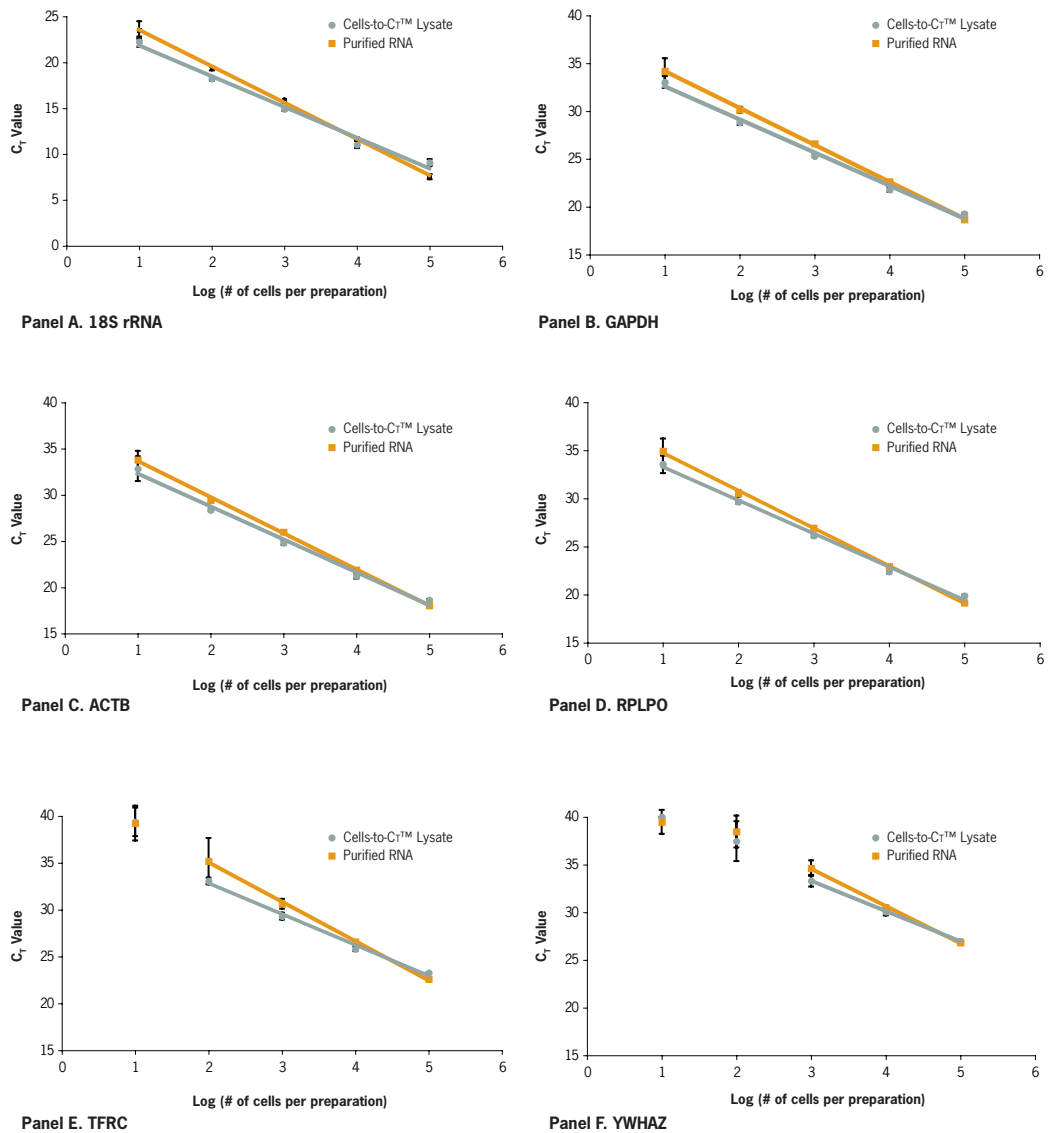


Figure 2. Performance of TaqMan® Gene Expression Cells-to-Ct™ Kit Lysates is Equivalent to or Better Than Purified RNA. Lysates or purified RNA samples generated from 10–100,000 HeLa cells were reverse transcribed, combined with TaqMan Gene Expression Master Mix, and loaded onto TaqMan Human Endogenous Control Arrays. Six of the 16 genes tested using the Endogenous Control Array are shown as representative results.

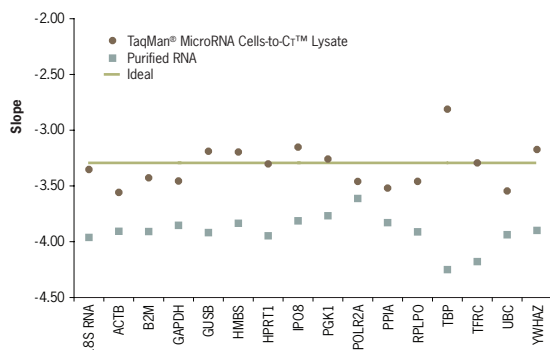
Results and Conclusions. The performance of lysates generated with the TaqMan Gene Expression Cells-to-Ct Kit was equivalent to or surpassed that of purified RNA. For many samples, especially those at low cell numbers, the sensitivity of assays using lysates was superior to that of purified RNA, likely due to sample loss during RNA isolation methods that use spin columns (Figure 2).

In addition to greater sensitivity at low cell numbers, lysis efficiency is maintained at high cell numbers as shown by the slopes of the data, which have a value close to the ideal of -3.3 (Figure 3A). Poor efficiency (slope >3.5) seen with the traditional RNA isolation samples is likely due to inefficient cell lysis and sample recovery. A squared linear correlation coefficient close to 1.0 is observed for all assays (Figure 3B).

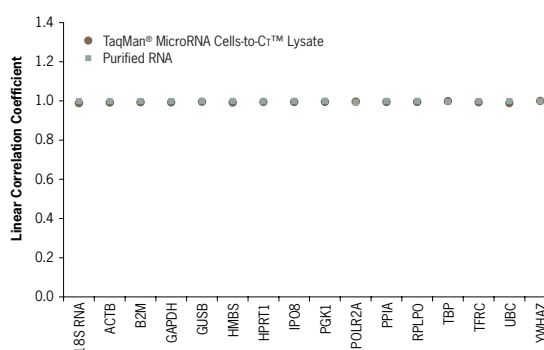
The TaqMan Gene Expression Cells-to-Ct Kit and TaqMan Arrays provide a complete workflow for optimal simplicity and speed with robust results customers expect from Applied Biosystems products. For a complete list of available TaqMan Gene Signature Arrays, visit taqmanarray.appliedbiosystems.com

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Panel A. Slope



Panel B. Squared Linear Correlation Coefficient

Figure 3. The Slope and Squared Linear Correlation Coefficients for TaqMan® Gene Expression Cells-to-Ct™ Kit Lysates Are Near Ideal. Lysate or purified RNA generated from 10–100,000 HeLa cells was reverse transcribed, combined with TaqMan Gene Expression Master Mix, and loaded onto Human Endogenous Control Panel TaqMan Arrays. Slopes of C_T values versus log(cell number) (A) and squared linear correlation coefficients (R²) (B) were calculated for each assay over the range of cell numbers.

TaqMan® Arrays for Easy and Robust Gene Expression Analysis

Although individual TaqMan Gene Expression Assays may be used, TaqMan Arrays (micro fluidics cards pre-loaded with up to 384 TaqMan Gene Expression Assay primer/probes) provide the ultimate solution for simplified real-time PCR analysis, as these arrays:

- Require minimal amounts of sample
- Allow 1–8 samples to be tested in parallel with 12–384 TaqMan Gene Expression Assay targets (including manufacturing controls)
- Do not require any liquid-handling robotics or complex pipetting

Simply load the sample and TaqMan Gene Expression Master Mix into each of the eight loading ports of a TaqMan Array using a standard pipette, centrifuge the TaqMan Array to dispense the sample mix into the individual wells, and cycle the reactions on an Applied Biosystems 7900HT Fast Real-Time PCR System with the TaqMan Array block for accurate and reproducible gene expression data. Since each TaqMan Array requires just a few minutes to set up, hundreds of genes can be analyzed with minimal hands-on time.

TaqMan Gene Signature Arrays are pre-designed TaqMan Arrays that target genes related to specific diseases or pathways and that are designed to facilitate drug discovery, disease research, and pathway analysis. The genes are chosen from pathway analysis tools, published articles, and collaborator and customer input. Alternatively, customers may design their own TaqMan Custom Arrays, choosing from over 50,000 inventoried TaqMan Gene Expression Assays. (For more information, visit taqmanarray.appliedbiosystems.com.)

ORDERING INFORMATION	P/N	SIZE
TaqMan® Gene Expression Cells-to-Ct™ Kit	AM1728	100 lysis rxns, 500 PCRs (20 µL)
	AM1729	400 lysis rxns, 2000 PCRs (20 µL)
TaqMan® Cells-to-Ct™ Control Kit	4386995	100 rxns
TaqMan® Gene Signature Arrays	Varies*	Varies†
TaqMan® Custom Arrays	Varies*	Varies
*2 cards for Endogenous Control Arrays & 4 cards for other Gene Signature Arrays		
Applied Biosystems 7900HT Fast Real-Time PCR System	Varies	1 instrument
Applied Biosystems 7900HT TaqMan® Low Density Array Upgrade	4329012	1 each†
†Includes a TaqMan® Array block, sealer, centrifuge buckets and adaptors, and a chemical install kit		
RELATED PRODUCTS		
TaqMan® Gene Expression Assays—Inventoried	4331182§	250 rxns
TaqMan® Gene Expression Assays—Made-to-Order	4351372§	360 rxns
§Visit www.allgenes.com to search for and order specific assays.		

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