TaqMan® Gene Expression Cells-to-Ct™ Kit

A complete workflow for real-time RT-PCR without RNA purification

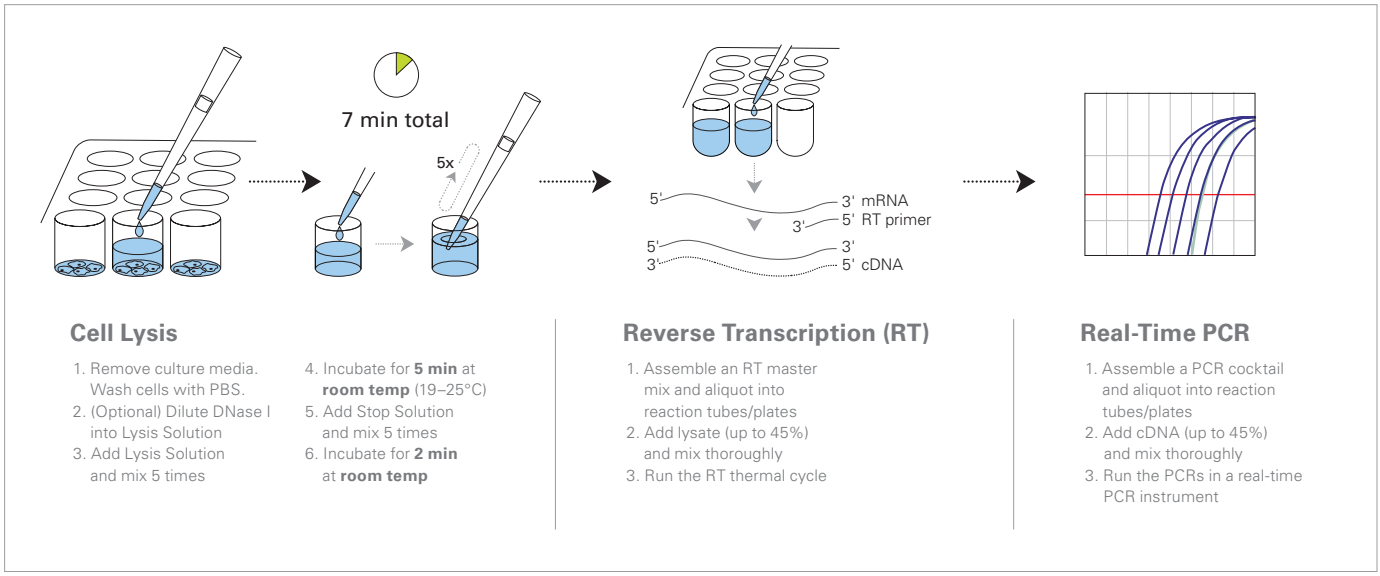

Figure 1. Samples are Ready for RT-PCR in Just 7 Minutes. The TaqMan® Gene Expression Cells-to-Ct™ Kit requires only 7 minutes at room temperature to release nucleic acids into a cell lysate solution. No centrifugation is needed, and the solution is compatible with RT and real-time PCR (optional DNase treatment included).

- Complete solution—Optimized workflow includes cell lysis reagents with gDNA removal, RT enzyme mix, buffer, and new TaqMan® Gene Expression Master Mix
- Fast—7-minute sample prep, including DNase treatment, at room temperature
- Easy—Lyse samples in a tube or directly in culture plates
- Robust—Perform gene expression analysis on 10–100,000 cells per sample; results equivalent to those from purified RNA
- Efficient—Contains sufficient reagents to generate 500 real-time PCR results from 100 starting samples

The TaqMan® Gene Expression Cells-to-Ct™ Kit makes it possible to perform expression analysis directly from cultured cells without RNA purification. This kit saves time and offers a simple workflow that is suitable for a few samples or can be easily incorporated into automated, high throughput applications.

Featuring a unique method for lysing cultured cells while removing genomic DNA and preserving the RNA integrity, 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. TaqMan primer/probe sets are sold separately.

Simple 7-Minute Sample Preparation: Part of a Complete Workflow

Whether you are using plates or tubes, the TaqMan Gene Expression Cells-to-Ct Kit, which uses the simple 7-minute sample preparation procedure outlined in Figure 1, is designed for 10 –100,000 cultured cells/sample. Cells are washed in PBS and lysed in solution for 5 minutes at room temperature; DNase treatment can be performed simultaneously. Lysis is terminated at room temperature by a 2-minute incubation with Stop Solution.

The TaqMan Gene Expression Cells-to-Ct Kit lysates are now ready for reverse transcription or storage at –20°C. 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 old-fashioned multi-step RNA isolation protocols, no heating, washing, or centrifugation steps are required. The kit greatly simplifies a laborious 30–60 minute process and reduces it to 7 minutes.

The TaqMan Gene Expression Cells-to-Ct Kit workflow enables unsurpassed gene expression evaluation with any of the >700,000 TaqMan Gene Expression Assays. This new kit has

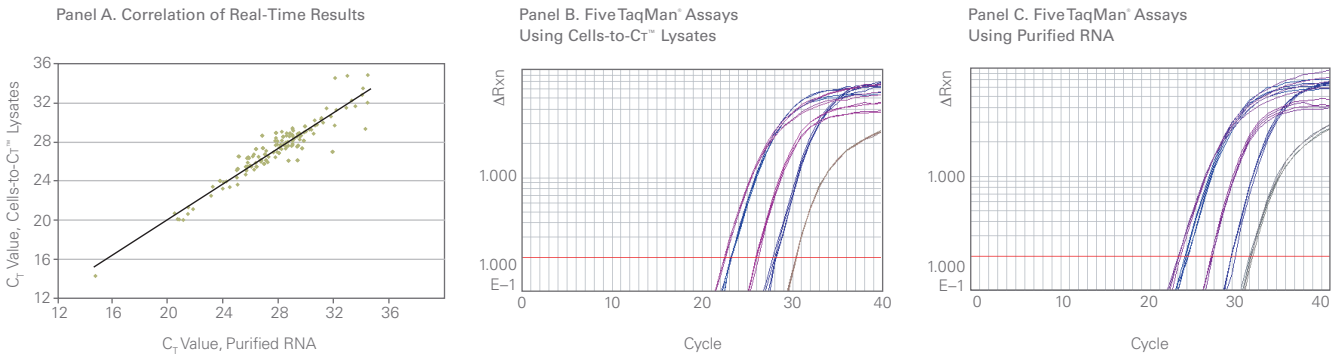

Figure 2. Correlation of Real-Time PCR Results Between TaqMan® Gene Expression Cells-to-CT™ Lysates and Purified RNA. TaqMan Gene Expression Cells-to-CT Kit lysates and purified RNA from HeLa cells were prepared in parallel and evaluated with 137 TaqMan® Gene Expression Assays on an Applied Biosystems 7900HT Fast Real-Time PCR System. (A) The C_T value obtained from the TaqMan Gene Expression Cells-to-CT Kit lysates is plotted against the C_T value for the same assay using purified RNA. $R^2 = 0.903$ for the data. Detailed amplification plots for selected assays from TaqMan Gene Expression Cells-to-CT Kit lysates (B) and purified RNA (C) are shown for 5 TaqMan assays (Hs00168310_m1, Hs00166169_m1, Hs00163311_m1, Hs00153368_m1, Hs00257518_m1).

been extensively tested for specificity with a broad selection of TaqMan Gene Expression Assays and shows performance equivalent to that obtained with purified RNA (Figure 2).

Achieve Unsurpassed Performance and Sensitivity

Unlike some competitor kits that limit the amount of lysate in the RT reaction to 5%, the TaqMan® Gene Expression Cells-to-CT™ Kit can accommodate 45% of the total RT reaction volume as cell lysate. Additionally, cDNA can comprise up to 45% of the real-time PCR reaction volume.

The large lysate volume in the optimized RT reaction, along with the large cDNA volume in the subsequent real-time PCR using the TaqMan® Gene Expression Master Mix, lead to maximum sensitivity. The master mix amplifies the target precisely and accurately, enabling the detection of small quantities of target, such as transcripts expressed at low levels.

The ability to detect limited target quantities was tested by mixing a constant number of human (HeLa) cells with various numbers of mouse (NIH 3T3) cells. The cell mixtures were prepared using the TaqMan Gene Expression Cells-to-CT Kit and assayed for a mouse-specific (Mm B2M) and human-specific (Hs TKT) gene. Comparative data were generated in the same manner with RNA purified by traditional methodology.

The data show that RNA from as few as 10 mouse cells was detectable in a background of RNA from 10,000 human cells (Figure 3). The ability of the TaqMan Gene Expression Cells-to-CT Kit to detect relative low abundance transcripts was equivalent to that of purified RNA; at low levels, it was superior.

Additionally, the performance of the TaqMan Gene Expression Cells-to-CT Kit was compared to competitor lysate kits and to purified RNA. Inputs of 100–100,000 cells/lysis reaction were examined. The sensitivity of the TaqMan Cells-to-CT Kit protocol was equivalent

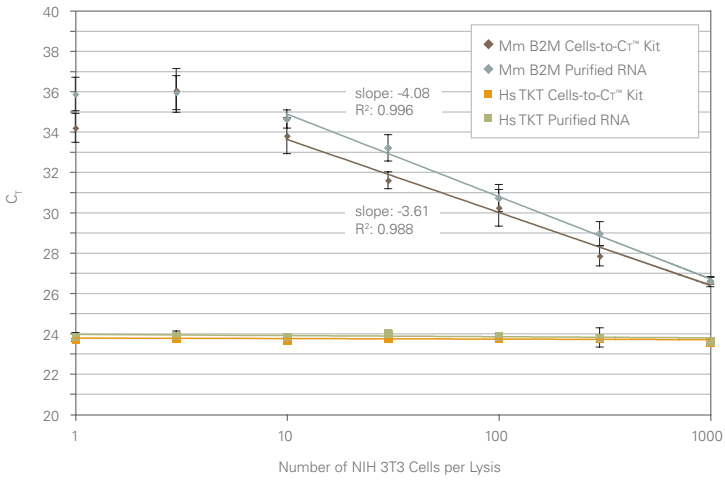

Figure 3. Detection of Limited Target Sequences with the TaqMan® Gene Expression Cells-to-CT™ Kit. Increasing amounts of NIH3T3 cells (mouse) were added to a constant 10,000 HeLa cells (human). These cells were processed in triplicate by the TaqMan Gene Expression Cells-to-CT Kit or purified using a RNA spin column method. All samples were reverse transcribed by the RT reagents provided in the kit at recommended volumes. Real-time PCR was conducted for mouse-specific (Mm B2M) and human-specific (Hs TKT) genes on all samples in triplicate reactions on a 7900HT Fast Real-Time PCR System.

to that obtained with purified RNA, and it surpassed competitor lysates (Figure 4). Furthermore, the lack of inhibition at high cellular inputs and the low variation among technical replicates demonstrate the reliability of this approach for gene expression studies using cultured cells.

Simple, Reliable siRNA Experiments

The growing interest in RNAi screening utilizing siRNA-mediated gene silencing is driving the need for innovative tools for this application. The TaqMan® Gene Expression Cells-to-CT™ Kit simplifies detection of siRNA-induced mRNA knockdown via qRT-PCR by eliminating the need for RNA purification without sacrificing the sensitivity and robustness required for reliable results.

In the experiment depicted below, HeLa cells were transfected with 20 different individual siRNAs or *Silencer*® GAPDH Positive Control siRNA. After incubation, samples were prepared for qRT-PCR with either the TaqMan Gene Expression Cells-to-CT Kit or by a traditional magnetic bead based RNA purification method, and gene silencing was subsequently measured using the corresponding TaqMan® Gene Expression Assay.

Samples prepared with the TaqMan Gene Expression Cells-to-CT Kit showed results equivalent to purified RNA across all targets (Figure 5), demonstrating the excellent performance of the TaqMan Gene Expression Cells-to-CT Kit in facilitating analysis of siRNA-mediated transcript knockdown.

Proven Performance, Proven Together

All components of the TaqMan Gene Expression Cells-to-CT Kit have been optimized for consistent and reliable performance. This removes the guesswork involved in assembling separate sample preparation, RT, and real-time PCR kits. And the TaqMan Gene Expression Cells-to-CT Kit has been validated with TaqMan Gene Expression Assays for added quality assurance.

The new TaqMan Gene Expression Cells-to-CT Kit provides sufficient reagents for 500 real-time PCRs from 100 starting samples (100 rxn kit).

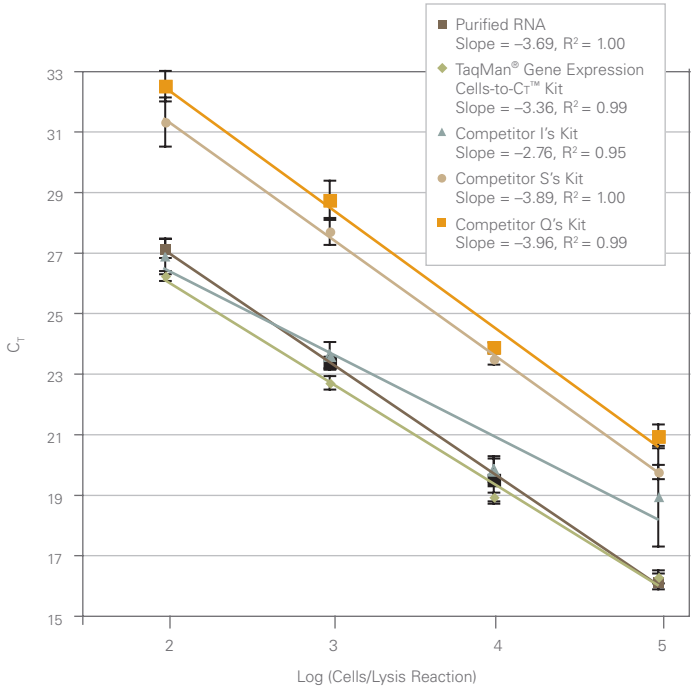

Figure 4. The Sensitivity of the TaqMan® Gene Expression Cells-to-CT™ Kit Versus Competing Technologies. HeLa cells (100–100,000) were prepared using either traditional RNA isolation, the TaqMan® Gene Expression Cells-to-CT™ Kit protocol, or other lysate methods from Competitors I, Q, and S. Reverse transcription reactions comprised the maximum recommended sample input for each kit, and real-time PCR was performed in triplicate using a PPIA TaqMan Gene Expression Assay.

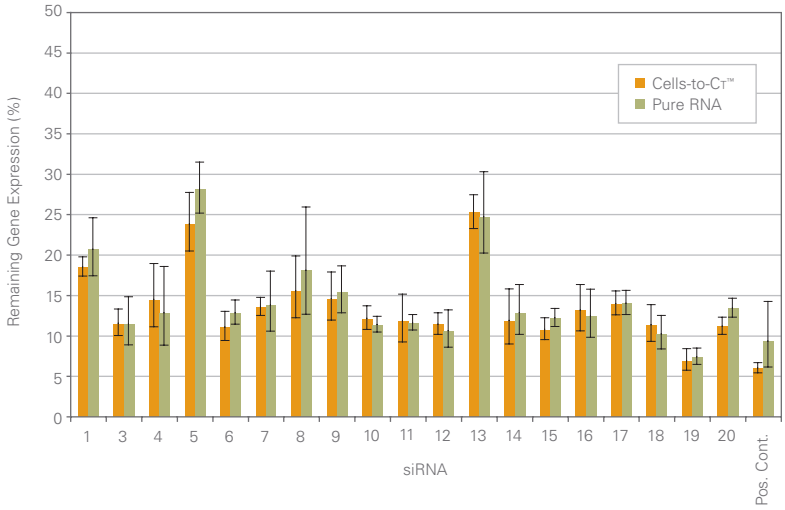

Figure 5. Performance of the TaqMan® Cells-to-CT™ Kit in Detecting siRNA-mediated Reduction of Gene Expression. HeLa cells were transfected with each of 20 different siRNAs or *Silencer*® GAPDH (h,m,r) Positive Control siRNA (AM4631) and grown for 48 hours in 96-well plates. Cells were processed in triplicate by the TaqMan Gene Expression Cells-to-CT Kit or by a magnetic bead purification method. All samples were reverse transcribed by the RT reagents provided in the kit at recommended volumes. Real-time PCR was conducted for appropriate genes in triplicate reactions on a 7900HT Fast Real-Time PCR System. Data are shown relative to negative control siRNA transfected samples.

ORDERING INFORMATION

Description	Size	Part Number
TaqMan® Gene Expression Cells-to-Ct™ Kit 100 lysis reactions with gDNA removal 100 cDNA synthesis reactions (50 µL) 500 PCRs (20 µL)	100 rxns	AM1728
TaqMan® Gene Expression Cells-to-Ct™ Kit 400 lysis reactions with gDNA removal 400 cDNA synthesis reactions (50 µL) 2,000 PCRs (20 µL)	400 rxns	AM1729
TaqMan® Cells-to-Ct™ Control Kit	100 rxns	4386995

Please inquire about bulk pricing.

RELATED PRODUCTS

Product Type	Description		Part Number
Accessories	MicroAmp™ 96- & 384-Well Optical Adhesive Film		4311971
	Nuclease-free Water (not DEPC-treated)		AM9938
Assays	TaqMan® Gene Expression Assays, for information go to **www.allgenes.com**		
Instruments	StepOnePlus™ Real-Time PCR System		4376600
	Applied Biosystems 7300 Real-Time PCR System		4351101
	Applied Biosystems 7500 Real-Time PCR System		4351104
	Applied Biosystems 7900HT Fast Real-Time PCR System with Standard 96-Well Block Module		4329003
	Applied Biosystems 7900HT Fast Real-Time PCR System with 384-Well Block Module		4329001
Kits	TaqMan® Cells-to-Ct™ Control Kit		4386995
Master Mixes	TaqMan® Gene Expression Master Mix	5 mL	4369016
		2 x 5 mL	4369514
		10 x 5 mL	4369542
Plates	MicroAmp™ Optical 384-Well Reaction Plate		4343370
	MicroAmp™ Optical 96-Well Reaction Plate		4316813
RNAi	*Silencer*® Pre-designed siRNA, standard purity	5 nmol	AM16708
	Silencer® Validated siRNA, standard purity	5 nmol	AM51331
	Silencer® Control siRNAs	5 nmol	Various
	Silencer® siRNA Libraries	Variable	Various
	siPORT™ *NeoFX*™ Transfection Agent	0.4 mL	AM4510

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