TaqMan® PreAmp Cells-to-Ct™ Kit

Extend Gene Expression Analyses without RNA Isolation

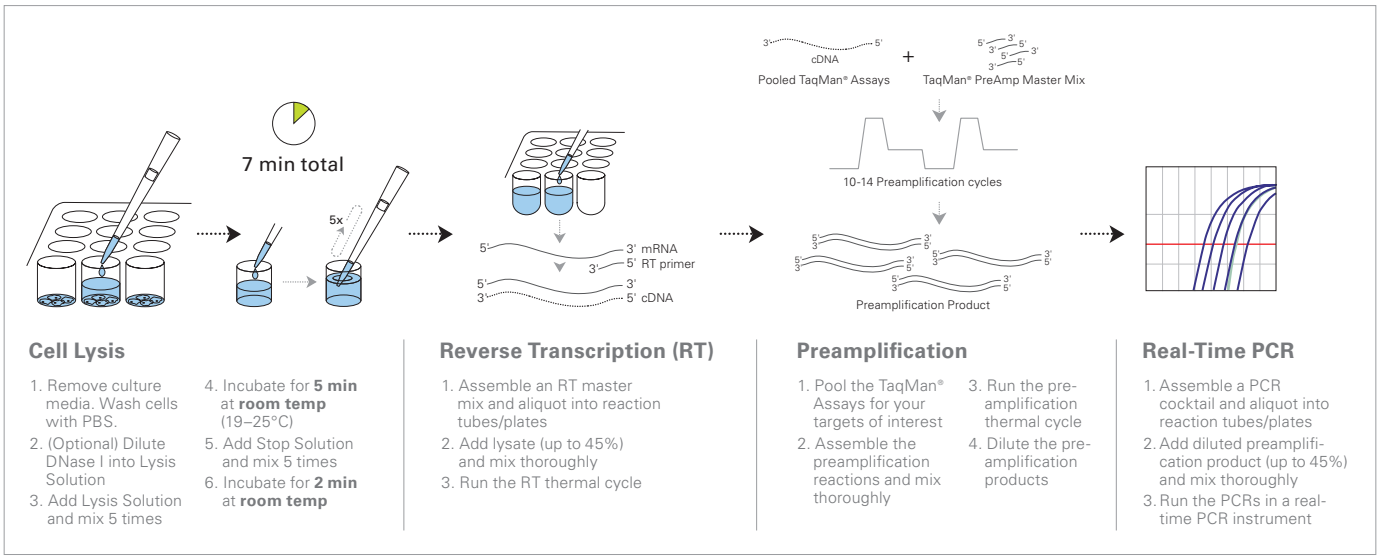

Figure 1. Samples are Ready for RT-PCR in just 7 Minutes. The TaqMan® PreAmp Cells-to-Ct™ Kit requires only 7 minutes at room temperature to release nucleic acids into a cell lysate solution. No centrifugation is needed, and the solution is compatible with RT, TaqMan® PreAmp Master Mix, and real-time PCR reagents.

- **Complete, validated solution**—Optimized workflow includes cell lysis reagents, DNase, RT reagents, PreAmp reagents, and TaqMan® Gene Expression Master Mix
- **Fast, simple and convenient**—Prepare samples in 7 minutes; eliminate tedious RNA isolation
- **Extend limited samples**—Increase real-time PCR potential by up to 64-fold; study up to 100 genes at once
- **Accurate results**—Uniform amplification without introducing bias
- **Robust performance**—Linear detection from 10 to 100,000 cells; results equivalent to purified RNA

The TaqMan® PreAmp Cells-to-Ct™ Kit makes it possible to perform gene expression analysis directly from limited or small numbers of cultured cells without RNA purification. For situations in which limited sample availability restricts the number of real-time PCR reactions, the TaqMan PreAmp Cells-to-Ct Kit enables experiments that would otherwise not be possible.

Complete, Validated Solution

Featuring a unique method for lysing cultured cells while removing genomic DNA and preserving the RNA integrity, the TaqMan PreAmp Cells-to-Ct Kit contains reverse transcription (RT) reagents for cDNA synthesis, TaqMan PreAmp Master Mix for pre-amplification of up to 100 gene targets, and TaqMan Gene Expression Master Mix for real-time PCR analysis.

Simple, Convenient 7-Minute Sample Preparation

The TaqMan PreAmp Cells-to-Ct Kit incorporates a simple 7-minute sample preparation procedure (Figure 1) for use with 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 concurrently. Lysis is terminated at room temperature by a 2-minute incubation with Stop Solution.

The TaqMan PreAmp Cells-to-Ct Kit lysates are now ready for reverse transcription or storage at –20°C. Because samples can be processed directly in culture plates (96 or 384 wells), sample handling and the potential for sample loss or transfer error are minimized, facilitating extension of limited samples. No heating, washing, or centrifugation is required; the kit greatly simplifies a traditionally time-consuming, labor intensive process and reduces it to 7 minutes.

Extension of Limited Samples—Increase Real-Time PCR Potential by up to 64-fold

The integration of the Cells-to-Ct™ technology with pre-amplification capability streamlines a multi-step

workflow while significantly extending (greater than 60-fold) the number of PCR reactions possible from precious samples, and increases the sensitivity of mRNA detection from small amounts of sample input.

The standard real-time PCR reaction for gene expression analysis starts with the reverse transcription of total RNA to cDNA using random primers and oligo dT, followed by real-time PCR using gene-specific primers and probes (Figure 2). With the TaqMan® PreAmp Cells-to-CT™ Kit, an intermediate amplification step (pre-amplification) between reverse transcription and real-time PCR is performed in which cDNA is enriched for up to 100 gene targets of the user's choice without amplification bias. The resulting pre-amplified reaction is diluted and serves as the starting material for the subsequent individual TaqMan® Gene Expression Assays using the TaqMan® Gene Expression Master Mix, included in the kit. The TaqMan PreAmp process effectively extends samples up to 64-fold, enabling analyses previously not possible from limited sample material.

Accurate Results—Equal Amplification without Introducing Bias

The ability to conduct reliable and uniform pre-amplification is critical for gene expression analysis from limited samples. While other methods are available to pre-amplify RNA or cDNA, they use non-specific priming to amplify the starting material, resulting in biased and inaccurate amplification of some transcripts over others. The TaqMan PreAmp Master Mix, part of the TaqMan PreAmp Cells-to-CT Kit, uses gene specific primers to pre-amplify cDNA resulting in unbiased amplification. The TaqMan PreAmp Cells-to-CT Kit demonstrates unbiased pre-amplification equivalent to purified RNA when compared across 100 different TaqMan Gene Expression Assays. As shown in Figure 3, the pre-amplification reaction

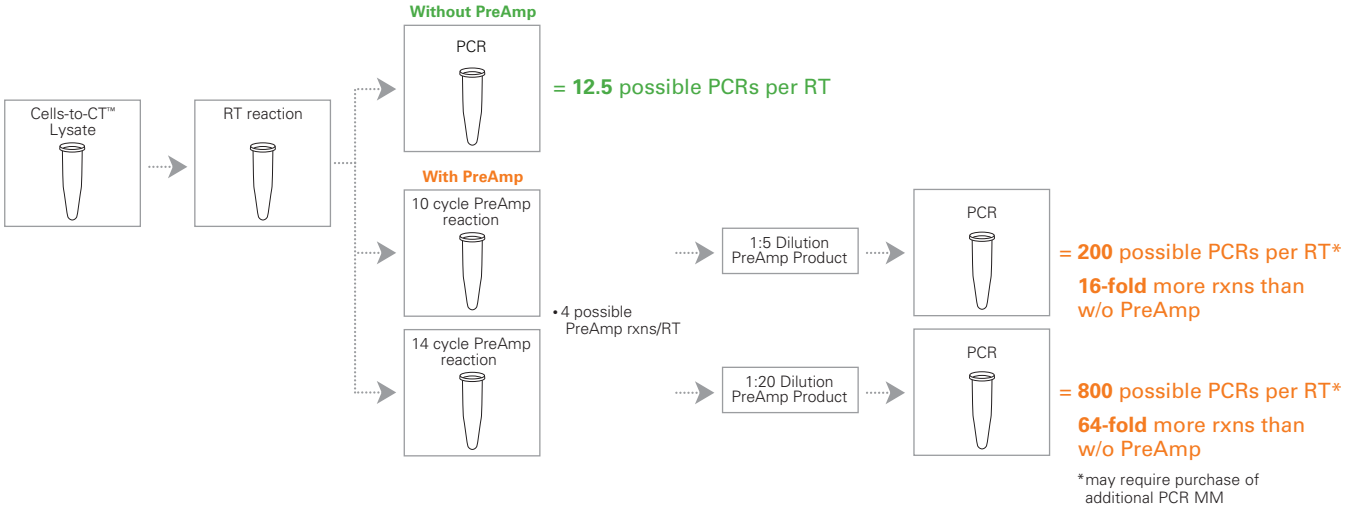

Figure 2. Pre-amplification Reaction Significantly Extends Limited Sample Amounts. Typically, a 50 µL reverse transcription reaction could generate up to 12.5 real-time PCR reactions containing 4 µL of cDNA each. With TaqMan® PreAmp Master Mix, cDNA is enriched for up to 100 genes, using an unbiased multiplex amplification step between reverse transcription and real-time PCR. The pre-amplification reaction is performed on reverse transcribed samples for 10 or 14 cycles, (depending on # of genes in multiplex reaction). The sample is diluted for singleplex real-time analysis. The pre-amplification significantly extends the amount of limited or precious samples available for subsequent analysis by singleplex real-time PCR.

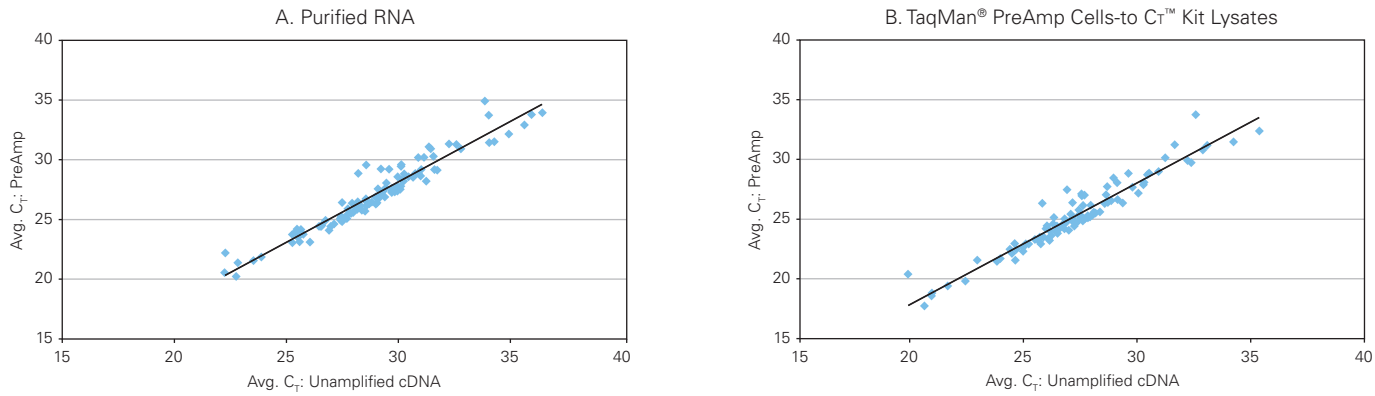

Figure 3. Pre-amplification of Cells-to-CT™ Lysates Does Not Introduce Bias. 10,000 HeLa cells were lysed using TaqMan® PreAmp Cells-to-CT™ Kit or underwent RNA purification using glass fiber filter columns. Reverse transcription reactions were performed using the provided reaction components and 100 different TaqMan® Assays were amplified in triplicate. The graphs show un-amplified cDNA on the X-axis and diluted pre-amplification product on the Y-axis. Graph A is purified RNA (R^2= 0.923, slope = 1.014) and Graph B is TaqMan PreAmp Cells-to-CT Kit lysates (R^2= 0.922, slope = 1.001).

does not introduce bias from Cells-to-CT™ lysates nor purified samples; ≥ 92% of the assays were within +/- 1.5 ΔΔ CT for pre-amplified vs. un-amplified cDNA from either purified RNA (A) or from Cells-to-CT™ Kit lysates (B).

Robust Performance—Results Equivalent to Purified RNA

Performance of the TaqMan® PreAmp Cells-to-CT™ Kit was evaluated with 100 different TaqMan® Assays utilizing Cells-to-CT™ lysates or purified RNA, either with or without pre-amplification.

As shown in Figure 4, the C_T values obtained with TaqMan PreAmp Cells-to-CT Kit lysates were equivalent to, and frequently better than C_T values obtained using purified RNA, either prior to (A) or after pre-amplification (B). The high level of performance from TaqMan PreAmp Cells-to-CT Kit lysates can be attributed to the preservation of RNA in the starting samples without loss from heating, transfer, or adsorption to matrices.

Linear Detection from 10 to 100,000 Cells

Each step of a workflow for profiling RNA must be robust to work from a few, or many cells. The TaqMan PreAmp Cells-to-CT Kit has been optimized to efficiently generate linearity across 5 logs of cellular input, from 10–100,000 cells per lysis reaction (Figure 5). Furthermore, to maximize sensitivity, the TaqMan PreAmp Cells-to-CT Kit allows up to 45% of the RT reaction volume to be comprised of sample lysate. The TaqMan PreAmp Cells-to-CT Kit provides maximal sensitivity derived from the large lysate volume used in the RT reaction, optimized RT reaction conditions, and sample pre-amplification prior to real-time PCR.

Proven Performance, Proven Together

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

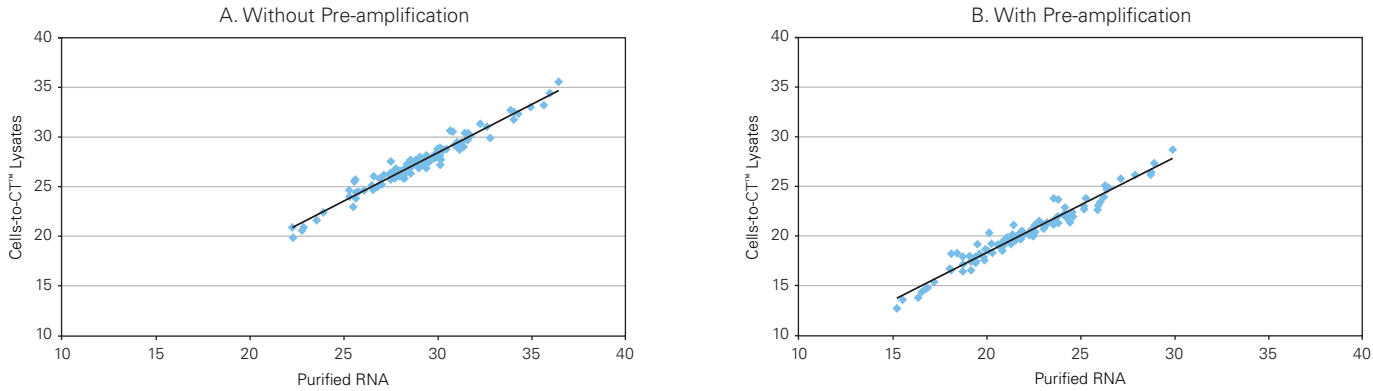

Figure 4. Performance of Cells-to-CT™ Lysates is Equivalent To Purified RNA Before and After Pre-amplification. 10,000 HeLa cells were processed by purifying RNA using glass fiber filter columns or lysed using the TaqMan® PreAmp Cells-to-CT™ Kit. Reverse transcription was performed using the provided reaction components and samples were analyzed for 100 individual targets directly (panel A, $R^2 = 0.959$, slope = 0.975), or with pre-amplification using pooled primers (panel B, $R^2 = 0.953$, slope = 0.960). The graphs display average C_T values of triplicate technical replicates for Cells-to-CT™ lysates (Y axis) versus purified RNA (X axis).

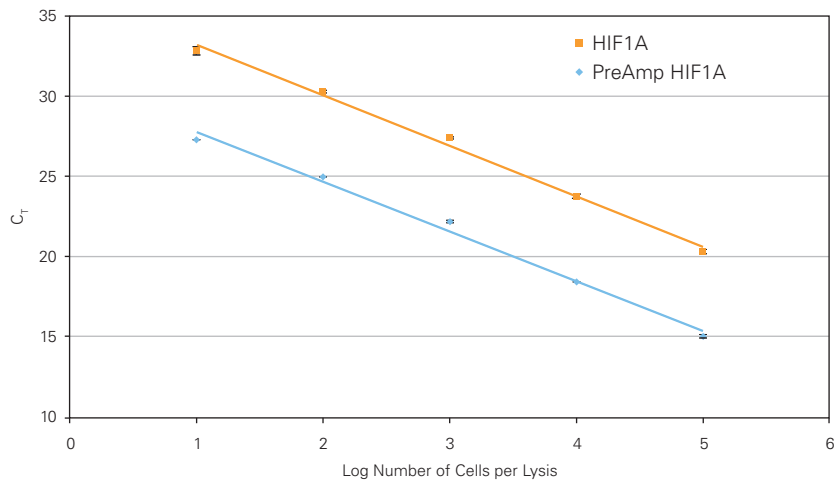

Figure 5. Linear and Sensitive Real-time RT-PCR Response Across a Wide Dynamic Range of Cell Input Using the TaqMan® PreAmp Cells-to-CT™ Kit. A dilution series of 10–100,000 HeLa cells was processed in triplicate with the TaqMan PreAmp Cells-to-CT Kit. The gene HIF1A (Assay ID Hs00153153_m1) was analyzed from cDNA (not pre-amplified, magenta line) or diluted pre-amplification product (blue line) in triplicate and averaged values were plotted as a function of input amount. For both templates, amplification was linear over 10–10^5 cells per lysis. The linear correlation coefficient was 0.9947 and 0.9915 for samples prepared without and with TaqMan PreAmp Cells-to-CT Kit, respectively.

ORDERING INFORMATION

Description	Size	Part Number
TaqMan® PreAmp Cells-to-CT™ Kit 40 lysis reactions with gDNA removal 40 cDNA synthesis reactions (50 µL) 40 pre-amplification reaction Up to 500 PCRs (20 µL)	40 lysis rxns 40 pre-amp rxns 500 real-time PCR rxns	4387299

Please inquire about bulk pricing.
For Research Use Only. Not for use in diagnostic procedures.

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 Applied Biosystems offers more than 750,000 TaqMan® Gene Expression Assays across eight species, the most comprehensive set of pre-designed Real-Time PCR assays available. All TaqMan® Gene Expression Assays have been designed using our validated bioinformatics pipeline, and run with the same PCR protocol, eliminating the need for primer design or PCR optimization. Custom TaqMan® Gene Expression Assays enable researchers to create new TaqMan assays by submitting sequences for any organism to target any site within a gene, across exon boundaries, or within an exon.		4331182
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, Standard 96-well Block		4329003
	Applied Biosystems 7900HT Fast Real-Time PCR System, Standard 384-well Block		4329001
Kits	TaqMan® Gene Expression Cells-to-CT™ Kit (100 lysis x up to 500 real-time PCR)		AM1728
	TaqMan® Gene Expression Cells-to-CT™ Kit (400 lysis x up to 2,000 real-time PCR)		AM1729
	TaqMan® Cells-to-CT™ Control Kit		4386995
Master Mixes	TaqMan® Gene Expression Master Mix	5 mL	4369016
		2 x 5 mL	4369514
		5 x 5 mL	4369510
		10 x 5 mL	4369542
		50 mL	4370074
	TaqMan® PreAmp Master Mix		4391128
Plates	MicroAmp® Fast Optical 48-Well Reaction Plate		4375816
	MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL		4366932
	MicroAmp® Optical 384-Well Reaction Plate		4343370
	MicroAmp® Optical 96-Well Reaction Plate		4316813

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