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Sample prep

Exceptional gene expression analysis directly from cell lysates: TaqMan Cells-to-C_T Express Kit outperforms alternatives

Summary

We show that the Invitrogen™ TaqMan™ Cells-to-C_T™ Express Kit is:

- **Exceptional**—The kit outperforms comparable 2-step workflows from Roche, Takara, Qiagen, NEB, and Bio-Rad.
- **Fast**—Cell lysates are prepared in five minutes and used directly in downstream reverse transcription (RT) and qPCR. The entire workflow can be executed in under 90 minutes and is easily automated.
- **Efficient**—The kit contains only 5 components to minimize pipetting steps and hands-on time. All required reagents for lysis, RT, gDNA removal, and qPCR are included.
- **Robust**—The kit is validated for 13 commonly used cell lines, producing results that are equivalent or superior to those obtained with purified RNA [1].
- **Sensitive**—The kit shows greater sensitivity, with lower C_t values, than comparable products from other suppliers.
- **Green**—The kit is fully REACH-compliant and produces no hazardous chemical waste [2]. It uses fewer pipette tips than other direct-lysis amplification or traditional column-based RNA purification workflows, reducing plastic waste by 80% or more, depending on the number of samples.

Introduction

Gene expression analysis has historically required the purification of RNA from cell lysates, which can be time-consuming and often involves the use of hazardous chemicals, prior to RT-qPCR. A number of products have emerged to simplify gene expression analysis of mammalian cells by allowing users to skip the RNA purification step and amplify targets directly from lysates. However, many of these workflows still employ hazardous chemicals and/or some form of post-lysis treatment, such as the addition of a stop solution or a heated incubation step, before the lysates can be added to the reverse transcription (RT) reaction. The Invitrogen™ TaqMan™ Cells-to-C_T™ Express Kit improves upon existing products by delivering fast and sensitive gene expression analysis directly from mammalian cell lysates without the use of a stop solution, post-lysis processing, or hazardous chemicals. Mammalian cells are lysed in 5 minutes using a nonhazardous, REACH-compliant lysis solution [2], and the resulting lysates are added directly to the RT reaction. The kit comes with all reagents needed for lysis, genomic DNA removal, reverse transcription, and qPCR, and includes a “no-RT” (no reverse transcriptase) negative control master mix to assess gDNA contamination.

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The exceptional processivity and inhibitor tolerance of the Invitrogen™ SuperScript™ IV VILO™ Master Mix included in the kit maximizes cDNA synthesis [3], while the double strand–specific Invitrogen™ ezDNase™ Enzyme effectively removes contaminating genomic DNA without risking cDNA digestion [4]. Additionally, the Applied Biosystems™ TaqMan™ Fast Advanced Master Mix permits fast PCR reaction conditions and maximal cDNA input to achieve sensitive and quantitative target amplification over a wide dynamic range [5]. This complete workflow exhibits exceptional performance and sensitivity compared to those of other kits designed for 2-step RT-qPCR analysis from cell lysates. The TaqMan Cells-to-C_T Express Kit has been validated for singleplex and duplex qPCR on Applied Biosystems™ QuantStudio™ Real-Time PCR Systems using VIC dye– and FAM dye–labeled TaqMan probes and has been shown to be compatible with many cell types and gene assays [6]. We show that the TaqMan Cells-to-C_T Express Kit delivers excellent performance and much greater sensitivity than other evaluated kits, while streamlining the workflow and reducing waste.

Materials and methods

HeLa cells were plated into 96-well plates at 10,000 cells per well in 150 µL DMEM with 10% FBS, and grown overnight in a humidified incubator at 37°C with 5% CO_2. The next day, four wells each were processed using the workflow and reagents included in the TaqMan Cells-to-C_T Express Kit (Figure 1), or following the procedures of the various lysis and two-step RT-qPCR kits from other manufacturers (Table 1).

Briefly, for all workflows, the cells were washed, then lysed in a lysis solution, and either immediately (for Thermo Fisher and Roche kits) or after lysate processing (for Bio-Rad, Qiagen, New England Biolabs, and Takara kits), the lysates were transferred into each kit's respective RT reactions using the maximum amount of lysate indicated. RNA in each lysate was reverse transcribed (+RT) and also treated using the negative control reaction without the reverse transcriptase (–RT). After incubation, the maximum amount of each RT reaction was added to the recommended qPCR master mix, and the *ACTB* gene was amplified on an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System using a TaqMan™ Gene Expression Assay (Hs03023880_g1), which can detect both reverse transcribed RNA and genomic DNA.

Each positive and negative RT reaction for each lysate was amplified in duplicate, resulting in 8 positive and 8 negative gene expression results for each workflow. The C_t values for each condition (n = 8) were averaged, and the standard deviations of the sample means were calculated. In cases where there was no measurable amplification after 40 cycles, the undetermined replicates were assigned a C_t value of 40. The effectiveness of genomic DNA removal was assessed for each kit by subtracting the average C_t values of reactions containing reverse transcriptase enzyme (+RT) from those of negative control reactions without reverse transcriptase enzyme (–RT). The propagated error of the difference of the averages was calculated. To quantify the ease of use of each workflow, the number of pipetting steps and reagents required to execute each workflow was counted.

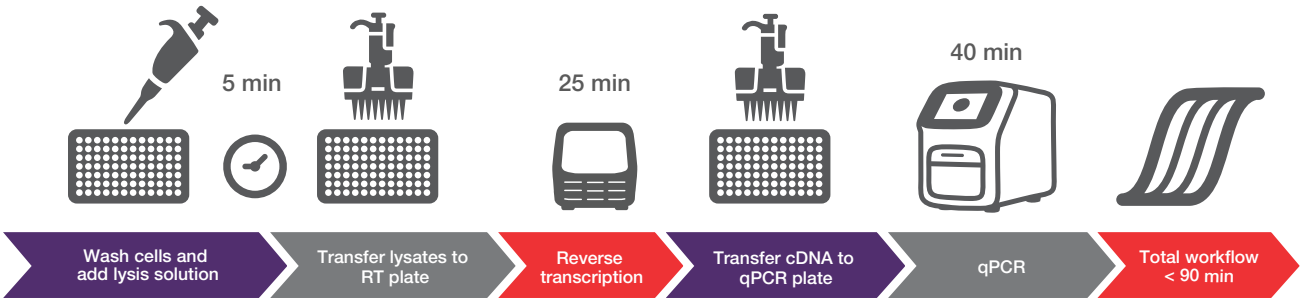

Figure 1. Workflow for the TaqMan Cells-to-C_T Express Kit.

Table 1. Lysis, reverse transcription, and qPCR kits evaluated in this comparison study.

	Direct lysis	Reverse transcription	qPCR
Thermo Fisher	**TaqMan Cells-to-C_T Express Kit**		
Roche	RealTime ready Cell Lysis Kit	Transcriptor First Strand cDNA Synthesis Kit	FastStart Universal Probe Master Mix
Bio-Rad	SingleShot™ Probes Kit for Cell Lysis and RT-qPCR		
Qiagen	FastLane Cell cDNA Kit		QuantiTect™ Probes PCR kit
New England Biolabs	Luna™ Cell Ready Lysis Module	LunaScript™ RT SuperMix Kit	Luna™ Universal Probe qPCR Master Mix
Takara	CellAmp™ Direct Probe RT-qPCR Kit		

Results

The TaqMan Cells-to-C_T Express Kit exhibited the highest sensitivity of all evaluated workflows, as reflected by the lowest C_t values for the targeted *ACTB* gene sequence (Figure 2), in part by permitting the highest input of cell lysate into the RT reaction and the highest input of RT reaction into the qPCR reaction, compared to the other kits (Figure 3). The TaqMan Cells-to-C_T Express Kit also had the simplest workflow, requiring the fewest reagents and pipetting steps (Figure 4). The effective removal of gDNA by ezDNase Enzyme is demonstrated by the large ΔC_t value obtained by subtracting the average C_t values of the positive (+RT) from those of the negative (–RT) RT reactions (Figure 5).

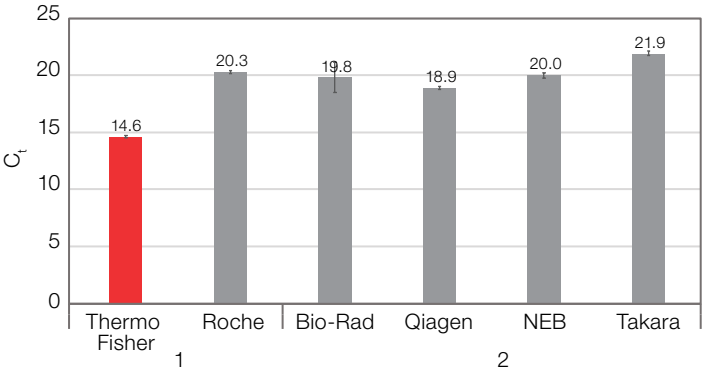

Figure 2. *ACTB* gene expression. Average C_t values for *ACTB* gene expression analysis using the TaqMan Cells-to-C_T Express Kit and other workflows. Group 1 kits do not require any lysate processing prior to the RT reaction. Group 2 kits require additional steps post-lysis prior to the RT reaction, such as a heated incubation step, addition of a lysis stop solution, or a separate gDNA digestion. Lower C_t values indicate greater sensitivity (n = 8; error bars represent one standard deviation of the sample mean).

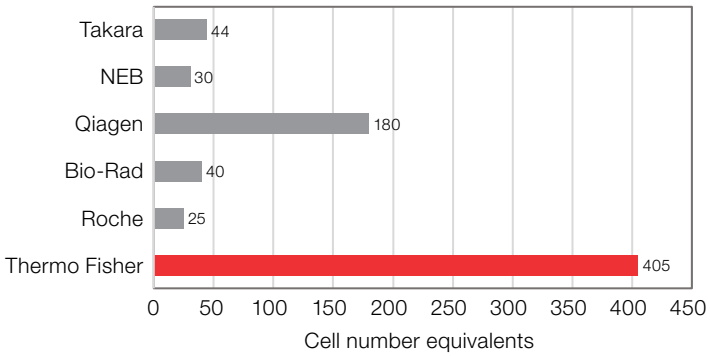

Figure 3. Comparison of cell number equivalents. Cell number equivalents using 10,000 cells per well, the maximum permitted input of lysate into the RT reaction, and the maximum permitted amount of RT reaction in the qPCR reaction for each workflow.

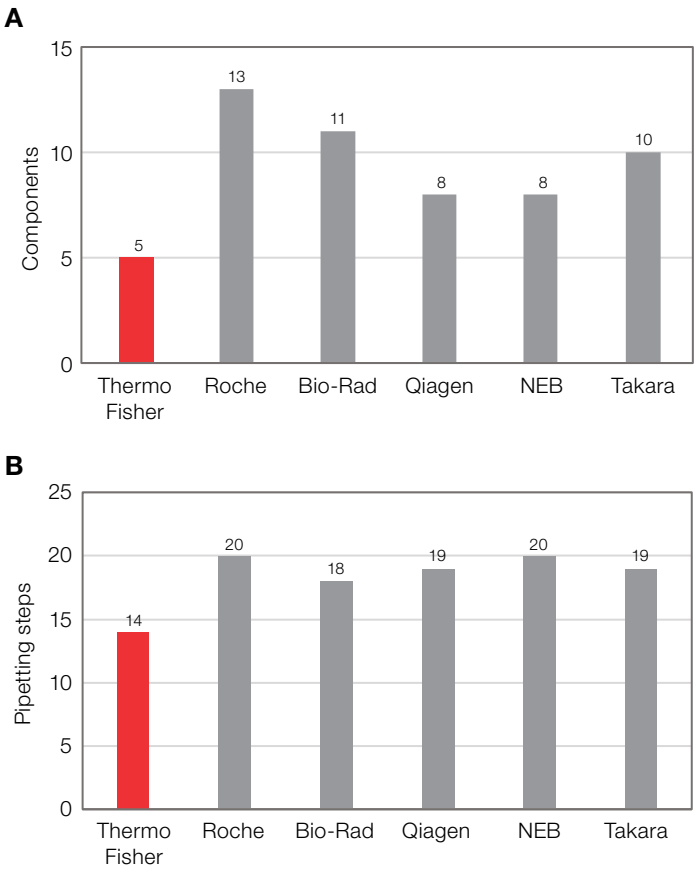

Figure 4. Comparison of workflows. (A) The number of components included in each kit (or kit combination) for each workflow. **(B)** The number of pipetting steps required to execute each workflow for one sample.

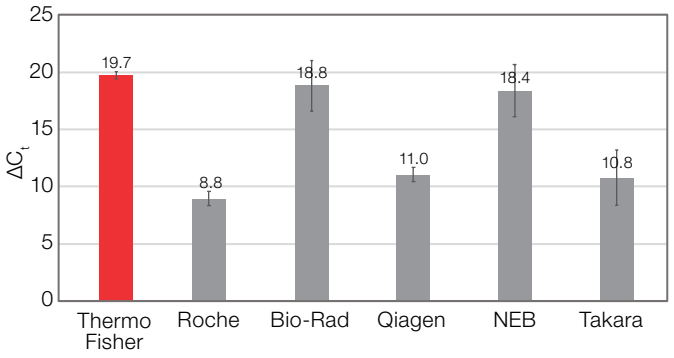

Figure 5. Comparison of ΔC_t obtained from *ACTB* amplification. ΔC_t is the difference between the average C_t values of reactions amplifying only gDNA (–RT) and those containing cDNA (+RT) and is indicative of the efficacy of DNase digestion. Larger ΔC_t values correlate with less gDNA detected in the sample relative to cDNA. Error bars represent the propagated error of the difference between the average C_t values of –RT and +RT reactions, where n = 8 for each.

Discussion

When compared side by side to other kits with similar workflows, the TaqMan Cells-to-C_T Express Kit reached C_t values 4 cycles earlier than the closest alternative kit, indicating 16-fold greater sensitivity. While the Cells-to-C_T kit's workflow allows for greater sample input than other kits tested, this factor alone can account for only a one-cycle C_t difference between the TaqMan Cells-to-C_T Express Kit and the kit with the next-highest sample input. The remarkable four-cycle C_t difference observed clearly highlights that it is not solely sample input volume, but rather the combined effect of the optimized workflow, the specially formulated Cells-to-C_T Express Lysis Buffer, the highly processive and inhibitor-tolerant SuperScript IV VILO Master Mix, and the performance-proven TaqMan Fast Advanced qPCR Master Mix that enables these superior results. Additional advantages of the TaqMan Cells-to-C_T Express Kit are its simple workflow, which minimizes hands-on time and pipetting steps while reducing plastic waste, and its REACH-compliant, nonhazardous reagents. In conclusion, this delivers an exceptionally user-friendly and sensitive solution for gene expression analysis directly from cell lysates.

References

1. Thermo Fisher Scientific (2023) TaqMan Cells-to-C_T Express Kit is compatible with multiple cell lines for gene expression analysis. **Application note**.
2. REACH is a regulation of the European Union created to improve the environment and protect human health. More information can be found at the **European Chemicals Agency**.
3. Thermo Fisher Scientific (2016) SuperScript IV VILO master mix for optimal RT-qPCR. **White paper**.
4. Thermo Fisher Scientific (2016) ezDNase Enzyme. **Product information sheet**.
5. Thermo Fisher Scientific (2018) TaqMan Fast Advanced Master Mix. **Product bulletin**.

Ordering information

Product	Quantity	Cat. No.
TaqMan Cells-to-C_T Express Kit	40 reactions	A57985
	100 reactions	A57986
	400 reactions	A57987
	2,500 reactions	A57988
TaqMan Cells-to-C_T Express Lysis Reagents (bulk lysis solution only)	2,500 reactions	A57989
TaqMan Fast Advanced Master Mix	1 mL	4444556
	5 mL	4444557
	2 x 5 mL	4444963
	5 x 5 mL	4444964
	10 x 5 mL	4444965
SuperScript IV VILO Master Mix with ezDNase Enzyme	50 reactions	11766050
	500 reactions	11766500
SuperScript IV VILO Master Mix	50 reactions	11756050
	500 reactions	11756500
ezDNase Enzyme	50 reactions	11766051
TaqMan Gene Expression Assay (FAM dye)	250 reactions/250 µL	4331182

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