ThermoFisher SCIENTIFIC

Real-time PCR

Accelerate cytochrome P450-mediated drug-interaction studies with the TaqMan Cells-to-C_T HepatoExpress Kit

Summary:

- **Fast**—gene expression results from primary hepatocytes in as little as 75 minutes
- **Robust**—performance comparable to column-purified RNA
- **Simple**—cell lysis, reverse transcription, and qPCR reagents all in one kit
- **Scalable**—adaptable to liquid-handling platforms for high-throughput processing

Introduction

Metabolism and toxicity studies are important aspects of drug discovery and development. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) specifically recommends that investigational drug candidates be evaluated for their effects on cytochrome P450 (*CYP*) gene expression at the mRNA level in human hepatocytes [1]. Sample preparation, specifically RNA purification, can be a significant bottleneck to evaluating large numbers of samples for gene expression analysis of *CYP* mRNA. One way to save time and increase throughput is to use what is known as a "direct lysis" kit. These kits permit interrogation of mRNA targets directly from crude lysates of cells without the need for RNA purification. While significant time savings can be achieved by using a direct lysis kit, many of the available kits either do not perform well on primary hepatocytes or have undesirable properties such as multiple reagents and pipetting steps, heated incubations, or hazardous chemicals.

The Invitrogen™ TaqMan™ Cells-to-C_T™ HepatoExpress™ Kit offers an effective, efficient, and nonhazardous direct lysis solution for plated hepatocytes. The workflow produces results comparable to those obtained using purified RNA and can be easily integrated into liquid-handling platforms for high-throughput applications. The kit includes Invitrogen™ SuperScript™ IV VILO™ RT Master Mix (and No RT master mix as a negative control), Applied Biosystems™ TaqMan™ Fast Advanced Master Mix, and Invitrogen™ ezDNase™ Enzyme for a complete gene expression analysis workflow in one box. Additionally, it features the Invitrogen™ HepatoExpress™ Lysis Solution and HepatoExpress™ LysisGuard™ reagent, which have been formulated to effectively lyse hepatocytes and stabilize samples. All components of the TaqMan Cells-to-C_T HepatoExpress Kit are nonhazardous and REACH compliant, and help provide an eco-friendly solution for hepatocyte RNA preparation. Furthermore, the inclusion of master mixes (instead of individual reaction components) minimizes the number of required pipetting steps and the amount of plastic tip waste. In this study, we demonstrate that the performance of the TaqMan Cells-to-C_T HepatoExpress Kit is comparable to that of a traditional silica column–based RNA extraction kit for examining induction of *CYP* mRNA in primary human hepatocytes after treatment with standard inducers.

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Materials and methods

Five lots of human primary hepatocytes (Thermo Fisher Scientific) were plated into 96-well plates at a density of either 80,000 (lots 1 and 2) or 70,000 (lots 3, 4, and 5) cells per well, on two different days. For each experiment, cells were allowed to adhere to the wells in a humidified 37°C incubator with 5% CO_2 for 4–6 hours before applying an overlay of 0.35 mg/mL Gibco™ Geltrex™ basement membrane matrix. Plates were returned to the incubator overnight. The following day, triplicate wells of each lot were treated with either 1 mM phenobarbital (PB), 50 µM omeprazole (OMP), 10 µM rifampicin (RIF), or 0.1% dimethyl sulfoxide (DMSO) as a vehicle control, and returned to the incubator overnight. After 24 hours, the medium was replaced with fresh dosing medium, and the plates were returned to the incubator for another 24 hours. The cell monolayers were then washed and processed using either the TaqMan Cells-to-C_T HepatoExpress Kit (Figure 1) or a spin column–based RNA purification kit.

The column-purified RNA was eluted into 50 µL of nuclease-free water to match the volume of the lysate obtained with the TaqMan Cells-to-C_T HepatoExpress Kit. Equal volumes of the lysates and purified RNA samples were reverse-transcribed with SuperScript IV VILO Master Mix at a final concentration of 10% of the total reaction volume. The reverse transcription reaction plate was incubated in an Applied Biosystems™ VeritiPro™ Thermal Cycler according to the user guide of the TaqMan Cells-to-C_T HepatoExpress Kit. The resulting cDNA was transferred to the qPCR reaction mix at 10% of the final qPCR reaction volume. Duplex qPCR was performed in duplicate for each sample, using a TaqMan Gene Expression Assay for *GAPDH* in combination with an assay for a *CYP* gene (either *CYP1A2*, *CYP2B6*, or *CYP3A4*) using TaqMan Fast Advanced qPCR Master Mix. The qPCR reactions were run on an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System using fast cycling conditions, and relative quantitation was performed using Applied Biosystems™ QuantStudio™ Design and Analysis Software v1.6.1.

Fold induction refers to the change in expression of the *CYP* mRNA in response to an inducer (phenobarbital, omeprazole, or rifampicin) compared to a control condition (DMSO). The *CYP* mRNA expression levels were first normalized within each well to those of the *GAPDH* gene, and then the normalized values from the inducer-treated samples were compared to the normalized values of the control samples. The fold induction was calculated by the $2^{-\Delta\Delta Ct}$ method [2], in which the DMSO-treated (vehicle control) samples were used as the reference.

Results

Fold-change values were well-correlated between samples processed with the TaqMan Cells-to-C_T HepatoExpress Kit and those processed with the spin-column kit, for all lots (Figure 2), as were the mean C_t values of replicate (non-normalized) qPCR reactions (Figure 3). The data demonstrate variation in mRNA fold-change values among the five lots of human primary hepatocytes in response to the inducer treatments, as well as some variability within the biological replicate wells per treatment, as might be expected. However, the mRNA induction response for all three of the targeted *CYP* genes is consistent between samples processed with the TaqMan Cells-to-C_T HepatoExpress Kit and those processed with the spin-column purification kit.

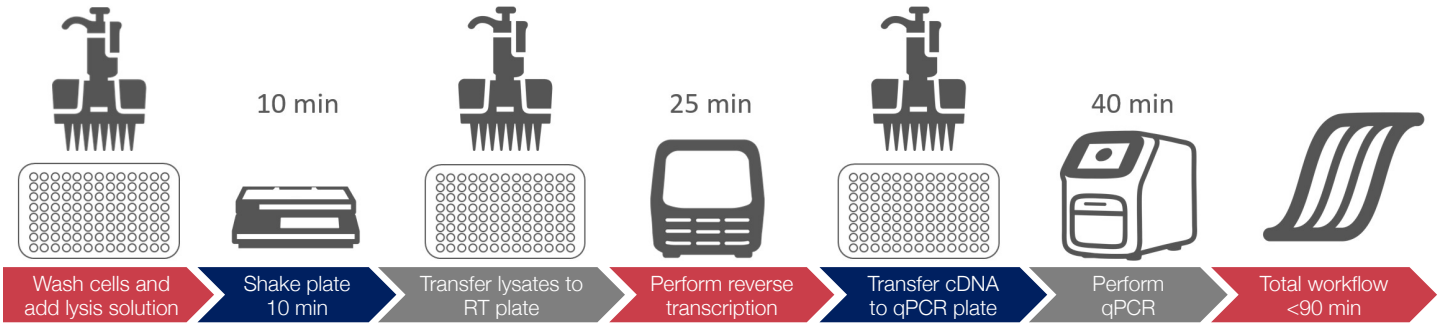


Figure 1. The TaqMan Cells-to-C_T HepatoExpress Kit workflow. RNA preparation is completed in only 10 minutes using a novel lysis buffer with added ezDNase enzyme and LysisGuard reagent. The entire workflow from plated cells to qPCR results is accomplished in as little as 75 minutes. All reagents to perform the workflow are included in the kit except for cell washing solution and Applied Biosystems™ TaqMan™ Gene Expression Assays.

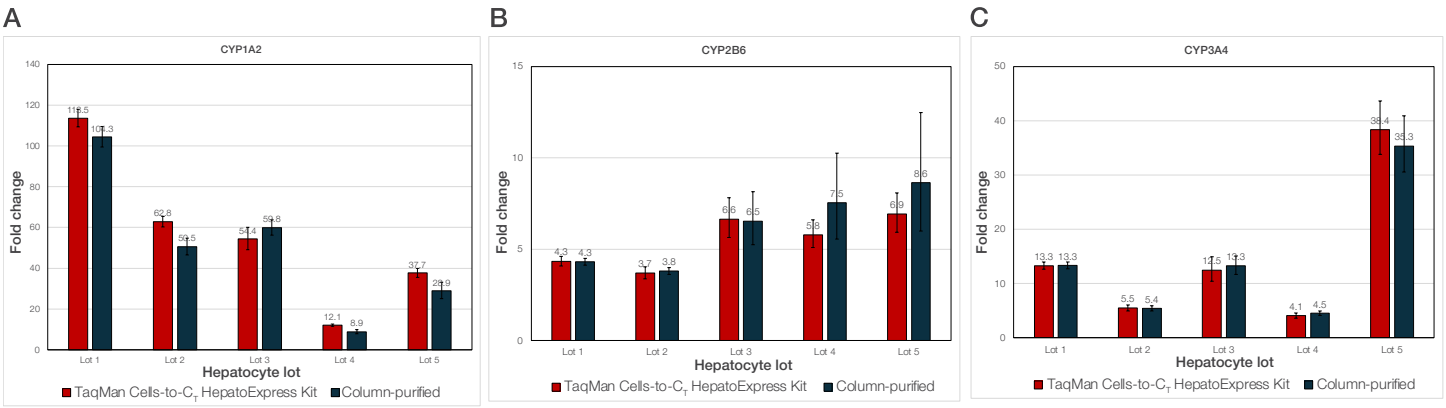
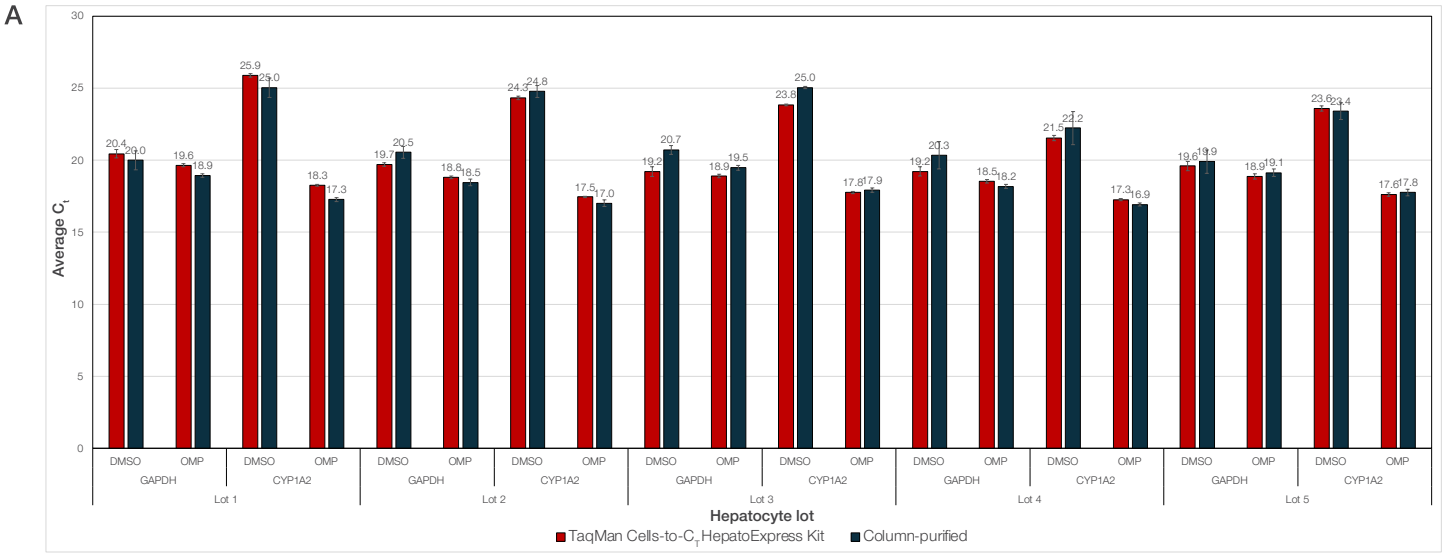
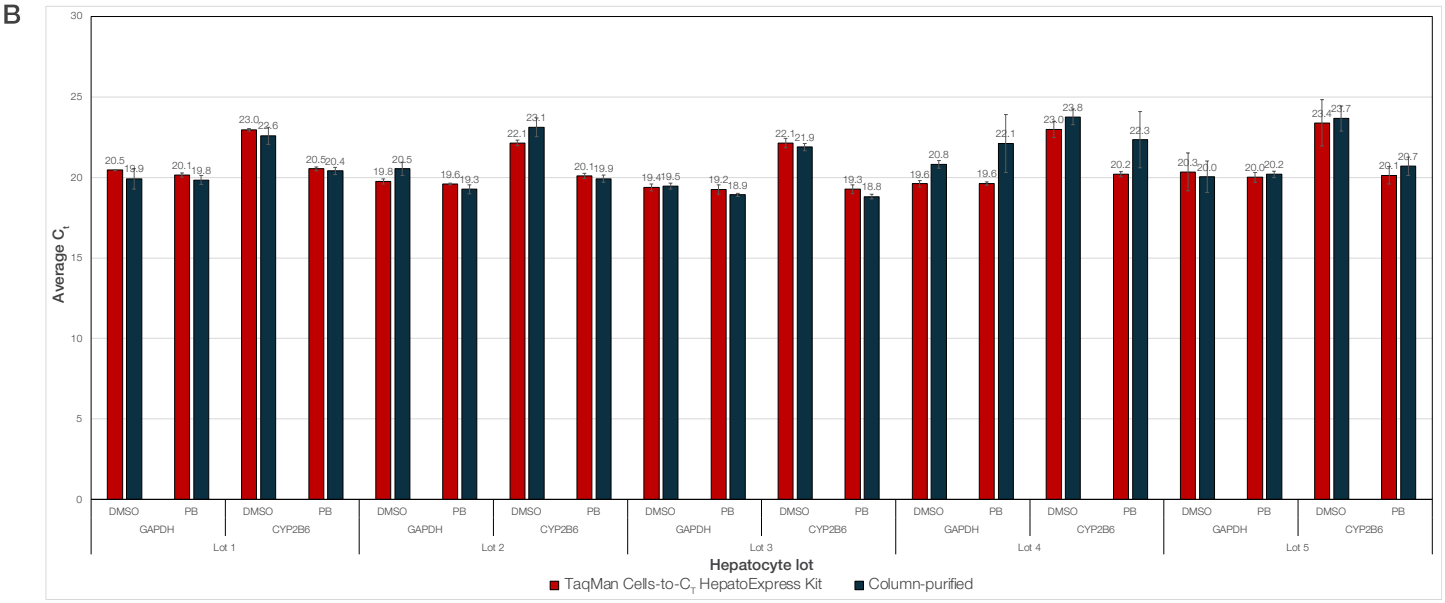


Figure 2. Induction of mRNA expression of *CYP* genes. (A) *CYP1A2*, **(B)** *CYP2B6*, and **(C)** *CYP3A4* fold-change values are shown for five lots of human hepatocytes processed with the TaqMan Cells-to-C$_T$ HepatoExpress Kit or an RNA spin-column purification kit. Error bars represent a 95% confidence interval for n – 1 degrees of freedom.





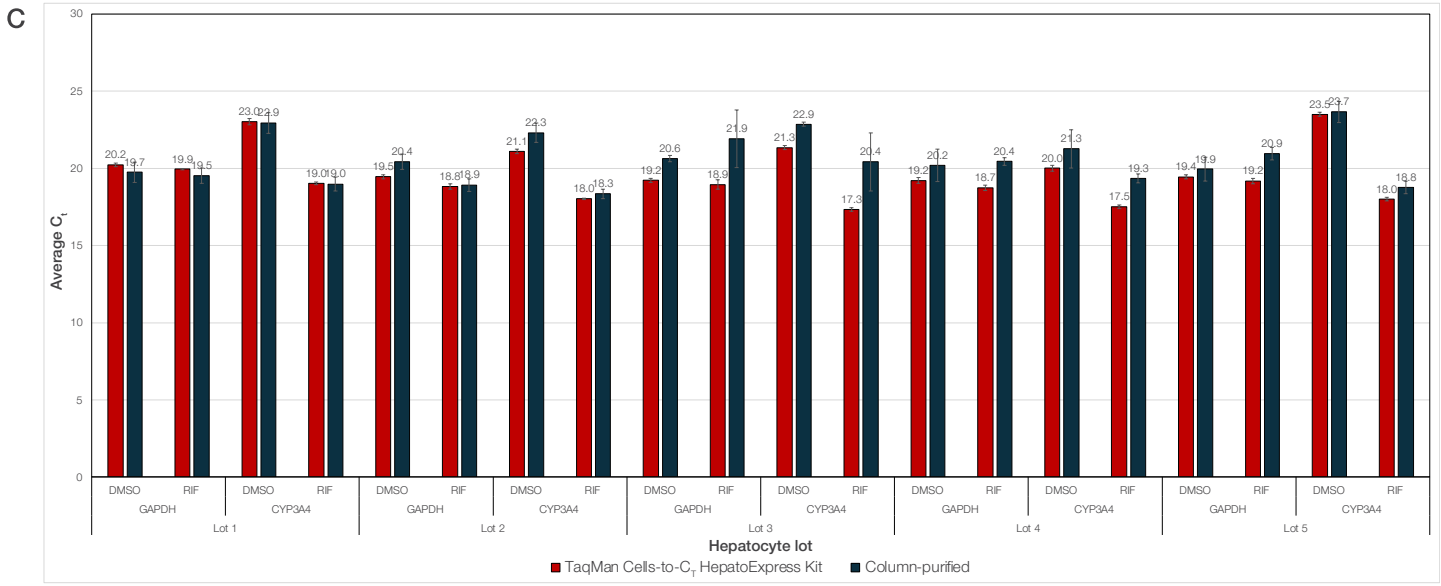


Figure 3. Comparison of average C_t values obtained for relevant *CYP* and *GAPDH* genes under the indicated treatment conditions. Shown are results after treatment with **(A)** omeprazole (*CYP1A2*), **(B)** phenobarbital (*CYP2B6*), and **(C)** rifampicin (*CYP3A4*). Error bars represent the sample standard deviation for 6 replicates.

Discussion

In this study, we show that the TaqMan Cells-to-C_T HepatoExpress Kit helps deliver reproducible induction results for *CYP* genes, consistent with those obtained using traditionally purified RNA, in a fraction of the time. It should be noted that the level of response to the inducers is strongly influenced by plating format and cell density. These response levels cannot be expected to be replicated precisely by other labs using different plating protocols, even when using the same lots of cells and inducer concentrations [3]. Variability in the raw C_t values may reflect well-to-well differences in the plated hepatocytes, and raw C_t values obtained using the TaqMan Cells-to-C_T HepatoExpress Kit are frequently lower than those seen for column-purified RNA, reflecting greater sensitivity. However, the ΔC_t values of the normalized data are similar between the RNA preparation methods. When employing the $\Delta\Delta C_t$ method, slight differences in raw and normalized C_t values are amplified by the transformation to fold-change values. Nevertheless, comparable results in induction response are demonstrated between samples processed with the TaqMan Cells-to-C_T HepatoExpress Kit and those processed using the column-based purification kit.

The workflow of the TaqMan Cells-to-C_T HepatoExpress Kit requires very few hands-on steps and only 10 minutes for RNA preparation, and yields results equivalent to those obtained with to the much longer and more labor-intensive RNA spin-column purification process. The entire workflow (from monolayer washing to RT-qPCR data output) takes as little as 75 minutes. The nonhazardous and REACH-compliant reagents eliminate the need for (and associated cost of) hazardous waste disposal incurred by other methods involving phenol extractions, guanidinium-based lysis reagents, or spin columns. In addition, because few components and pipetting steps are required, pipette tip waste is minimized, and automating the workflow of the kit on any liquid handler to further increase throughput is a straightforward task. Hepatocyte researchers can now eliminate tedious, hazardous, costly, and time-consuming RNA purification steps from hepatocyte gene expression workflows and accelerate drug discovery with the TaqMan Cells-to-C_T HepatoExpress Kit.

References

1. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (2024) M12 Drug Interaction Studies: Guidance for Industry.
2. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25(4):402–408.
3. Toyoda H, Nozue A, Nishida Y et al. (2025) Considerations for developing CYP induction assays in hepatocytes: Insights from a multilaboratory study. *Eur J Cell Biol* 104(3):151497.

Ordering information

Product	Quantity	Cat. No
TaqMan Cells-to-C_T HepatoExpress Kit	40 reactions	A65788
	100 reactions	A65789
	400 reactions	A65790
Lysis reagents only	2,500 reactions	A65791

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