Cells-to-CT™

The TaqMan™ Cells-to-CT™ HepatoExpress™ kit eliminates RNA isolation for CYP450 induction studies

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Introduction

Drug metabolism studies are an essential element of drug discovery and development. Sample preparation, specifically RNA purification, can be a significant bottleneck to processing large numbers of samples for CYP interactions using RT-qPCR. A simpler option is to skip RNA purification altogether using a direct lysis method. Reagent kits are available that permit amplification of targets directly from crude lysates of cultured cells without the need for RNA purification. With these "direct lysis" kits, cells are lysed in the culture plate wells and then reverse transcribed and amplified by RT-qPCR. Unfortunately, many of these direct lysis kits require multiple reagents and pipetting steps, heated incubations, employ hazardous chemicals, or are not optimized for hepatocyte cells.

The Invitrogen™ TaqMan™ Cells-to-CT™ HepatoExpress™ kit solves these problems by delivering a direct lysis workflow specifically designed for plated hepatocytes.The kit has a fast and convenient workflow and can be integrated into liquid handling platforms for high-throughput applications. The kit contains components for cell lysis and gDNA removal as well as SuperScript™ IV VILO™ RT Master Mix for reverse transcription and TaqMan™ Fast Advanced Master Mix for qPCR, enabling a complete gene expression analysis workflow. All kit components are non-hazardous and REACH-compliant.

In this study, we compared the results obtained using the TaqMan™ Cells-to-CT™ HepatoExpress™ kit to those obtained using a traditional silica column-based RNA extraction for use in a CYP450 induction experiment with previously untested human primary hepatocyte lots.

Materials and Methods

Two lots of previously untested human hepatocytes were plated in a collagen-coated 96-well plate at a density of 80,000 cells per well with a 0.35 mg/mL GelTrex™ basement membrane matrix overlay. Triplicate wells for each lot were treated with either 1 mM Phenobarbital (CYP2B6 inducer), 50 µM Omeprazole (CYP1A2 inducer), 10 µM Rifampicin (CYP3A4 inducer), or 0.1% DMSO (vehicle control). After 24 hours, the medium was replaced with fresh dosing medium, and the plates were returned to the incubator for another 24 hours. Cell monolayers were then washed and processed using either the TaqMan™ Cells-to-CT™ HepatoExpress™ kit and workflow (**Figure 1**) or a spin-column RNA purification kit. The column-purified RNA was eluted into 50 µL of nuclease-free water to match the volume of the TaqMan™ Cells-to-CT™ HepatoExpress™ lysate.

Equal volumes of the lysates and purified RNA samples were reverse transcribed using Superscript™ IV VILO™ Master Mix. Duplex qPCR was performed using the TaqMan™ Fast Advanced qPCR Master Mix using TaqMan™ gene expression assays targeting the GAPDH housekeeping gene (VIC-labeled) and a relevant CYP450 gene (FAM-labeled). qPCR reactions were cycled on an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System using fast cycling conditions. Relative quantitation analysis was performed according to the QuantStudio™ Design & Analysis Software v1.6.1. Fold induction values were calculated by the $2^{-\Delta\Delta CT}$ method [1] using threshold cycle values generated by the qPCR system.

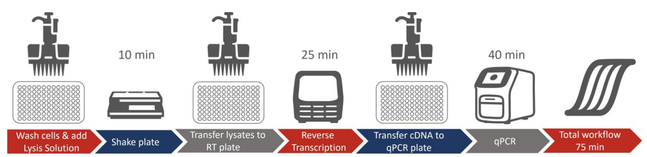
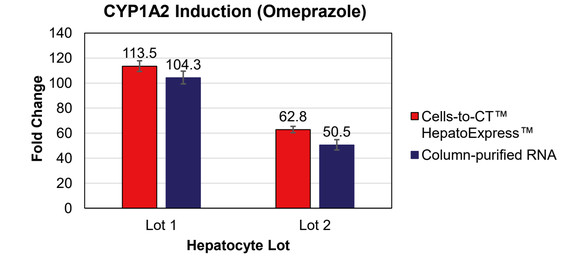
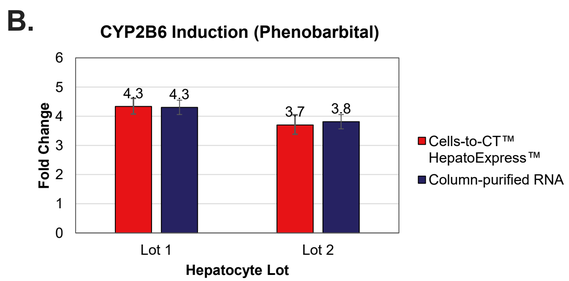


Figure 1. TaqMan™ Cells-to-CT™ HepatoExpress™ workflow. The TaqMan™ Cells-to-CT™ HepatoExpress™ kit contains all reagents necessary for hepatocyte cell lysis and gene expression analysis. The user only needs to supply TaqMan™ gene expression assays. Results can be obtained in approximately 90 minutes.

Results

CYP450 induction values obtained using either the TaqMan™ Cells-to-CT™ HepatoExpress™ kit or a column purification kit for RNA preparation were compared (**Figure 2**). Induction values between the two methods were found to be comparable.





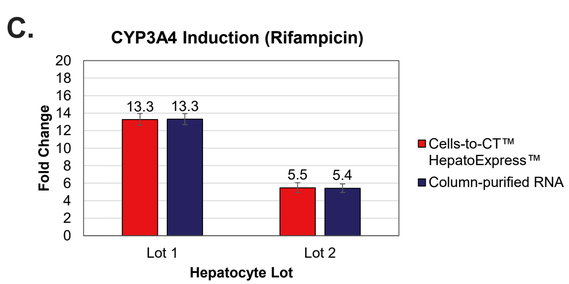
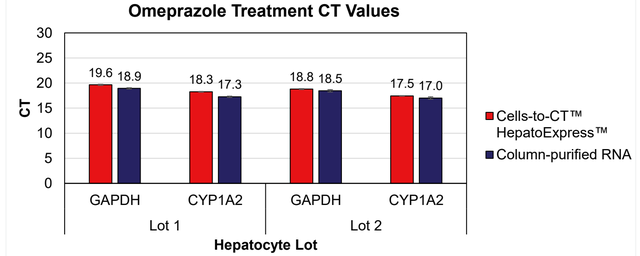
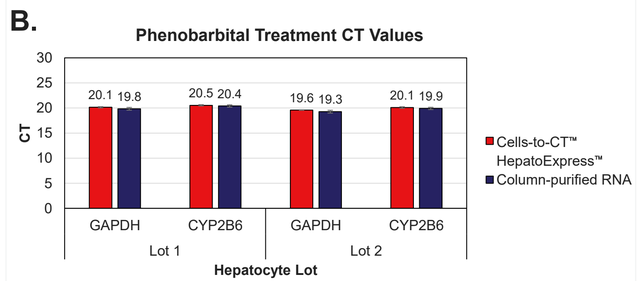


Figure 2. Fold induction values for (**A**) CYP1A2 (Omeprazole-treated) (**B**) CYP2B6 (Phenobarbital-treated), and (**C**) CYP3A4 (Rifampicin-treated) for two lots of previously untested human hepatocytes processed with TaqMan™ Cells-to-CT™ HepatoExpress™ or a column-purification method. Error bars represent 95% confidence intervals for 3 replicate samples.

Average CT values obtained using either the TaqMan™ Cells-to-CT™ HepatoExpress™ kit or a column purification kit for RNA preparation were compared (**Figure 3**). CT values between the two methods were found to be equivalent.





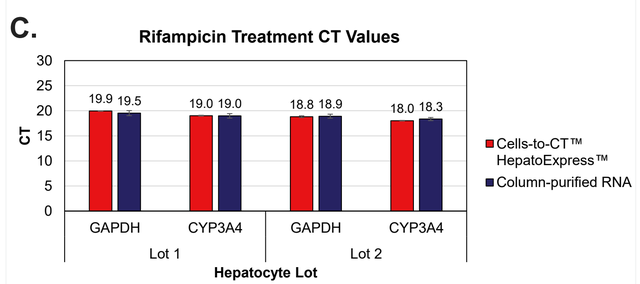


Figure 3. Comparison of average CT values obtained for relevant CYP450s and GAPDH under indicated treatment conditions: (**A**) Omeprazole (CYP1A2), (**B**) Phenobarbital (CYP2B6), and (**C**) Rifampicin (CYP3A4) for two lots of previously untested human hepatocytes processed with TaqMan™ Cells-to-CT™ HepatoExpress™ or a column-purification method. Error bars represent the standard deviation for 3 replicate samples.

Conclusions

In this study we assessed the suitability of using the TaqMan™ Cells-to-CT™ HepatoExpress™ kit for analysis of CYP450 induction using human primary hepatocytes and prototypical CYP450 inducers. The results of this study demonstrate that the TaqMan™ Cells-to-CT™ HepatoExpress™ kit produces induction values and raw CT values that are consistent with those obtained using a traditional column purification method for RNA isolation. Furthermore, the fold-change and CT values obtained using the TaqMan™ Cells-to-CT™ HepatoExpress™ kit showed minimal variation between replicate samples indicating efficient and effective sample processing. These findings underscore the reliability and efficiency of the TaqMan™ Cells-to-CT™ HepatoExpress™ kit, making it a robust alternative to the labor-intensive traditional RNA purification methods for CYP450 induction studies.

Discussion

The ability to process large numbers of samples quickly and efficiently is essential for accelerating the process of drug discovery. Sample preparation, specifically RNA purification, is a significant bottleneck to streamlining high-throughput workflows such as CYP450 mRNA induction studies. In this study, we show that the TaqMan™ Cells-to-CT™ HepatoExpress™ kit delivers fast and accurate CYP450 induction data consistent with that obtained using traditionally purified RNA. TaqMan™ Cells-to-CT™ HepatoExpress™ reagents are non-hazardous and REACH compliant, eliminating the need for (and associated cost of) hazardous waste disposal incurred by other methods (e.g. phenol extractions, guanidinium-based lysis reagents, spin-columns). Plastic pipette tip waste reduction is accomplished by the inclusion of master mixes for the reverse transcription and qPCR reactions and the elimination of a stop solution from the workflow. Because few components and few pipetting steps are required, automating the workflow on any liquid handler to further increase throughput is a simple and straightforward task, meaning that the TaqMan™ Cells-to-CT™ HepatoExpress™ kit can significantly speed up research for drug development and biomarker discovery. Eliminate tedious RNA purification steps from your hepatocyte gene-expression analysis workflows and get straight to results with the TaqMan™ Cells-to-CT™ HepatoExpress™ kit.

References

1. Livak, K. J, Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402-8. doi: 10.1006/meth.2001.1262.

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