ThermoFisher SCIENTIFIC

Sample prep

TaqMan Cells-to-C_T Express Kit is compatible with multiple cell lines for gene expression analysis

Introduction

The Invitrogen™ TaqMan™ Cells-to-C_T™ Express Kit enables reverse transcription and qPCR directly from cell lysates without the need to purify RNA. The TaqMan Cells-to-C_T Express Kit streamlines the gene expression analysis of cultured cells by eliminating the use of a stop solution that is common in conventional Invitrogen™ Cells-to-C_T™ kits. Lysates from the TaqMan Cells-to-C_T Express Kit are not only **as** sensitive as purified RNA, but often times more sensitive than purified RNA. This increased sensitivity has been demonstrated across multiple cell lines from which prepared RNA yields lower threshold cycle (C_t) values when the TaqMan Cells-to-C_T Express Kit is used compared to RNA isolated by column purification.

Materials and methods

Each of the cultured cell lines of interest (Table 1) were collected and washed in PBS. A 10-fold serial dilution of cells were prepared in PBS from 10^5 to 10 cells per 5 µL of PBS. Equal volumes (5 µL) of each dilution were used in the workflow of both the TaqMan Cells-to-C_T Express Kit and a commercially available spin column–based RNA purification kit, and extractions were done in duplicate for each cell concentration.

The protocol for the TaqMan Cells-to-C_T Express Kit was followed as outlined in the user guide. Briefly, cells were serially diluted in PBS, and a 5 µL aliquot of each dilution was dispensed into a plate and 50 µL of Express Lysis Solution premixed with Invitrogen™ ezDNase™ Enzyme was added directly to the cells. The commercially available RNA purification kit protocol was followed as instructed, starting with 5 µL of the same cell dilutions and eluting into 55 µL. The reverse transcription (RT) reactions of both protocols were carried out with equal volumes of either lysates or purified RNA, to allow for volume normalization.

The Invitrogen™ SuperScript™ IV VILO™ Master Mix that is provided in the TaqMan Cells-to-C_T Express Kit was used for RT for both sets of samples to prepare cDNA. In addition, the Applied Biosystems™ TaqMan™ Fast Advanced Master Mix, also provided in the TaqMan Cells-to-C_T Express Kit, was used for the qPCR reaction for both sets of samples to yield C_t values.

invitrogen

Table 1. Cell lines compatible with the TaqMan Cells-to-C_T Express Kit.

Cell line	Source species	Source tissue	Cell type
3T3	*M. musculus*	Embryo	Fibroblast
A549	*H. sapiens*	Lung	Epithelial carcinoma
CHO-K1	*C. griseus*	Ovary	Epithelial-like
HCT-116	*H. sapiens*	Large intestine; colon	Epithelial
HEK-293	*H. sapiens*	Kidney; embryo	Epithelial
HeLa	*H. sapiens*	Cervix	Epithelial adenocarcinoma
Gibco™ HepaRG™ Cells	*H. sapiens*	Liver	Hepatocytes
HepG2	*H. sapiens*	Liver	Hepatocellular carcinoma
Huh-7	*H. sapiens*	Liver	Hepatocellular carcinoma
Jurkat	*H. sapiens*	Peripheral blood	T lymphoblast
K562	*H. sapiens*	Bone marrow	Chronic myeloid leukemia
MCF-7	*H. sapiens*	Breast; mammary gland	Epithelial
Neuro 2A	*M. musculus*	Brain	Neuroblast
Raji	*H. sapiens*	Burkitt's lymphoma	B lymphocyte
SK-N-AS	*H. sapiens*	Brain	Neuroblast

To determine the performance of the TaqMan Cells-to-C_T Express Kit across multiple cell lines, an Applied Biosystems™ TaqMan™ Gene Expression Assay was used for qPCR, which was run simultaneously on the resultant cDNA from each of the cell lines. A 384-well plate was filled with the qPCR mixture and loaded with the cDNA, then run on an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System.

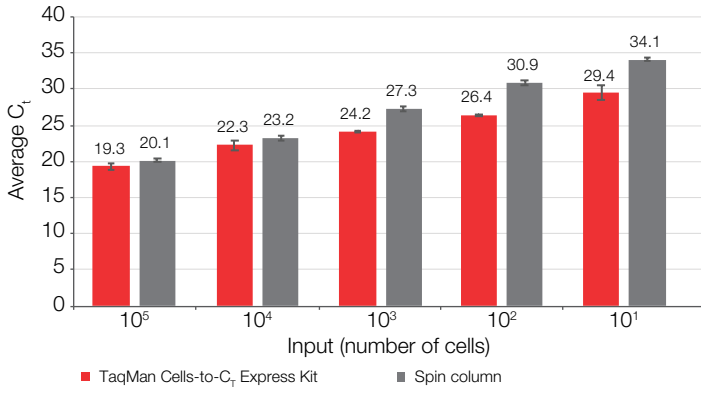

Figure 1. Average C_t values from Raji cells using the TaqMan Cells-to-C_T Express Kit or using a commercially available column-based RNA purification kit, using the input number of cells as shown.

Results

Each cell line was evaluated for compatibility with the TaqMan Cells-to-C_T Express Kit using gene expression analysis based on C_t values. We deemed compatibility when the C_t value from the commercially available column-based RNA purification kit was subtracted from the C_t value from the TaqMan Cells-to-C_T Express Kit to yield $\Delta C_t \leq 1$. The C_t values from Raji cells treated with the TaqMan Cells-to-C_T Express Kit were lower than those from Raji cells treated with the commercially available column-based RNA purification kit (Figure 1).

Standard curves were generated from the qPCR for each of the cell inputs, and the slopes of these graphs were used in the determination of the dynamic range of the cell lines with the TaqMan Cells-to-C_T Express Kit. Using SK-N-AS cells and targeting the gene for ß-actin, RNA prepared with the TaqMan Cells-to-C_T Express Kit outperformed the column-purified RNA, with a slope of –3.446, compared to that of –4.297 for the column-purified RNA (Figure 2A). Given that the optimal slope for the standard curve is –3.3, the data show that the TaqMan Cells-to-C_T Express Kit is not only a faster alternative for gene expression analysis but also often a more effective method for this dynamic range of cell input. This trend was observed for the commonly used cell lines listed in Table 1, with the RNA prepared from TaqMan Cells-to-C_T Express Kit outperforming the column-purified RNA by having both lower C_t values and more optimal slopes of the standard curves (data not shown).

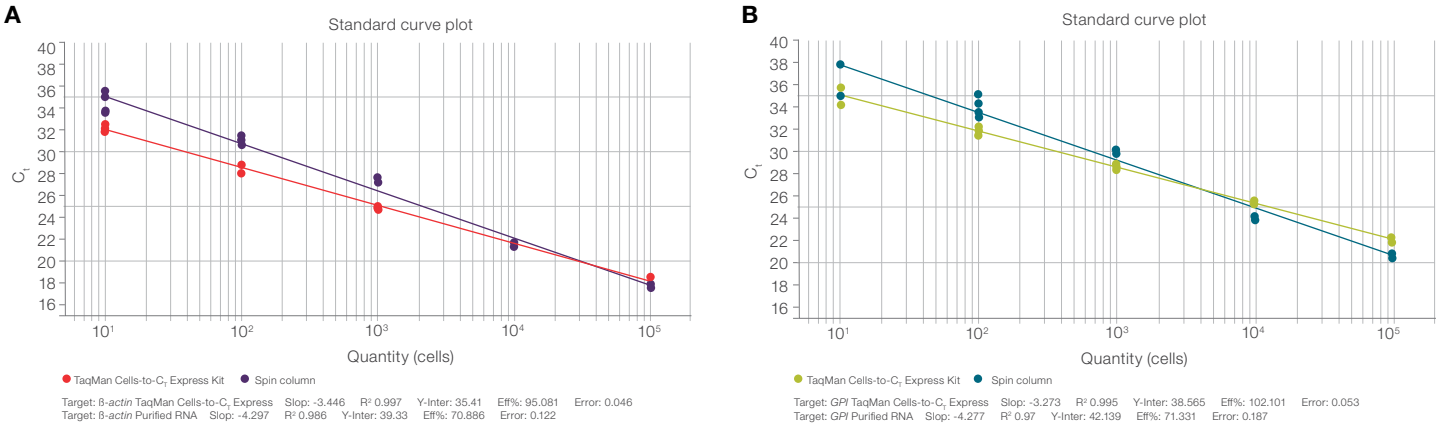

Figure 2. Standard curve of the C_t values for the SK-N-AS cell line. TaqMan Cells-to-C_T Express Kit and column purification were used for housekeeping genes **(A)** *β-actin* and **(B)** *GPI*. Duplicate sample preparation reactions, for both lysates and purified RNA, and duplicate qPCR reactions are shown. For each quantity of cells, there are four data points; however, some of the data points are overlapped so are not discernible.

The analysis was conducted with an additional housekeeping gene (*GPI*) to confirm consistency in the performance of the TaqMan Cells-to-C_T Express Kit with the various cell lines (data not shown). Similar trends were observed for *GPI* expression, with the C_t values obtained from the RNA prepared from TaqMan Cells-to-C_T Express Kit passing the criterion ($\Delta C_t \leq 1$) when tested against column-purified RNA. The slope of the curve from the TaqMan Cells-to-C_T Express Kit was closer to the ideal –3.3 (i.e., it was –3.273) than that of the curve generated with the commercially available column-based purification kit (–4.277) (Figure 2B).

Figures 2A and 2B illustrate a trend seen across the cell lines tested in Table 1, in which a larger difference in C_t values is evident at lower cell inputs (not all data shown). This phenomenon can be explained by the loss of RNA with the necessary transfers of the RNA product of the commercially available RNA purification kit; some of the RNA is lost to the column. The direct addition of the lysis buffer to the plated cells using the TaqMan Cells-to-C_T Express Kit reduces the loss of RNA, therefore providing greater sensitivity than column-purified RNA.

Conclusions

The elimination of the stop solution step in the TaqMan Cells-to-C_T Express Kit allows users to perform reverse transcription directly on the lysates, significantly increasing the efficiency of RNA preparation. The workflow for the TaqMan Cells-to-C_T Express Kit accelerates gene expression analysis of cultured cells without sacrificing sensitivity or specificity.

The efficiency of RNA preparation using the TaqMan Cells-to-C_T Express Kit was confirmed over a variety of cell lines. Gene expression analysis using RNA prepared from this kit was compared to RNA purified from a commercially available spin column kit, and the comparison indicates equivalent or lower C_t values from samples prepared from the TaqMan Cells-to-C_T Express Kit. The lower performance of spin columns could be caused by the loss of RNA that can occur with low sample input. However, at higher cell inputs, equivalent performance was observed (similar C_t values), as spin columns are typically more efficient with higher sample input. This study shows the efficiency of the TaqMan Cells-to-C_T Express Kit over a large dynamic range with a variety of cell lines.

Authors
Elizabeth Thomas, Cindy Boardman, and Laura Chapman
Thermo Fisher Scientific

Ordering information

Description	Quantity	Cat. No.
TaqMan Cells-to-C_T Express Kit	40 reactions	A57985
	100 reactions	A57986
	400 reactions	A57987
	2,500 reactions	A57988
TaqMan Gene Expression Assay	XS (75 reactions/75 µL), made to order	4448892
	XS (75 reactions/75 µL), inventoried	4453320
	S (360 reactions/360 µL), made to order	4351372
	S (360 reactions/360 µL), inventoried	4331182
	M (750 reactions/750 µL), made to order	4351370
	L (2,900 reactions/967 µL), made to order	4351368

Learn more at **thermofisher.com/cellstoct**