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Direct cDNA synthesis from single spheroids and primary cell samples

Key findings

Both the SuperScript IV CellsDirect cDNA Synthesis Kit and SuperScript IV Single Cell/Low-Input cDNA PreAmp Kit provide:

- The ability to work with low-input samples, including single 3D spheroids and primary cells
- Direct reverse transcription capabilities without RNA isolation that offer throughput and time-saving advantages
- High sensitivity for analysis of single cells and detection of rare transcripts

Abstract

Cell culture and transcriptome analysis are foundational techniques for life scientists exploring a variety of complex cellular processes. Traditionally, two-dimensional (2D) cell cultures have been used, which lack organizational complexity and limit the ability to predict cell behavior in complex physiological environments. To better mimic *in vivo* conditions, three-dimensional (3D) cell culture methods and primary cell types are increasingly being used in a variety of research fields from basic cell biology to cancer research and drug discovery. However, these sample types can present significant research challenges. Here, direct cDNA synthesis from unpurified single-spheroid and primary cell samples is demonstrated, using the **Invitrogen™ SuperScript™ IV CellsDirect™ cDNA Synthesis Kit** and the **Invitrogen™ SuperScript™ IV Single Cell/Low-Input cDNA PreAmp Kit**. cDNA synthesis yields were evaluated by performing real-time PCR (qPCR) and comparing gene expression levels to a more traditional method using samples processed with Invitrogen™ TRIzol™ Reagent. The SuperScript IV kits were shown to perform well with multiple cell types using both 2D cell cultures and 3D spheroids, and as few as 10 primary cells. The possibility to perform cDNA synthesis directly from cell lysates, without the need for nucleic acid purification steps, not only saves time and reagents, but also was shown to improve yield and reproducibility. The SuperScript IV kits should be considered by researchers working with low-input cell samples, especially if the detection of rare transcripts is required.

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Introduction

Cell culture is a fundamentally important technique that enables molecular analysis of complex biological processes under well-defined conditions. It is increasingly used in the life sciences, spanning many research applications from basic cell biology to cancer research and drug discovery. Traditionally, immortalized cell lines that have been cultivated on 2D surfaces or grown in suspension have been used for such research. However, 2D and suspension-based *in vitro* culturing of cells do not represent natural conditions, and this limits their ability to serve as a good predictor of cell behavior and response *in vivo*. This is especially true for cells that would normally exist in tissues and other 3D environments. For this reason, 3D culture methods that better mimic *in vivo* cell environments and provide greater translational potential are gaining acceptance [1,2].

One of the simplest and most common ways to culture cells in 3D is through the formation of multicellular spheroids. Formation of spheroids leverages the natural tendency of cells to aggregate and does not require special scaffolding. Thus, spheroids are a simple but valuable tool that allows scientists to model tumor growth and conduct high-throughput therapeutic studies [3].

Cells for spheroid formation can be derived either from immortalized cell lines or from primary tissues. Immortalized cell lines have been modified for unlimited proliferation, and therefore they are easily maintained, provide consistent experimental results, and are readily available from commercial sources. However, long-term culture may affect the genotype and function of cells, leading to a lack of the heterogeneity that is inherent to primary tissues. Another approach to represent *in vivo* conditions more accurately is to use cells extracted directly from human donor tissues or animal tissues. Such cells, extracted from a living source, are called primary cells. Despite their limited availability, primary cells offer significant advantages and ensure the most accurate replication of *in vivo* conditions [4,5]. A drawback is that they are often difficult to work with. Some of the undesirable properties of primary cells include their high sensitivity, low proliferation, tendency to differentiate, and cell senescence.

The relative pros and cons of different sample types must be considered not only when evaluating study results, but also when choosing the analysis methods for the study. Transcriptomics or gene expression analysis produce the primary types of data collected when exploring cellular processes at the molecular level. However, acquiring such data with high precision and reproducibility is not always easy.

Several key factors need to be considered to generate high-quality cDNA from single-cell or low-input samples:

- Minimal sample processing is recommended to avoid introduction of biases and sample loss during RNA extraction procedures.
- Lysis must be efficient to make all RNA accessible for reverse transcription (RT), although harsh lysis reagents may inhibit subsequent reactions.
- High-sensitivity enzymes are needed to detect low-abundance RNA targets.
- Adequate removal of genomic DNA is required to accurately quantify RNA targets.
- Workflows must be short and simple to avoid RNA losses that can occur from thermal degradation, the presence of RNases, or multiple purification steps.
- Robust enzymes with high inhibitor tolerance must be used for unpurified samples that contain cell debris and residual media (i.e., samples processed without nucleic acid extraction).

Here we demonstrate that the SuperScript IV CellsDirect cDNA Synthesis Kit and the SuperScript IV Single Cell/Low-Input cDNA PreAmp Kit address the above considerations and are well-suited for transcriptomic and gene expression applications with 3D cell cultures and primary cells, both of which are advantageous for translational research.

Materials and methods

Compared kits

Both the SuperScript IV CellsDirect kit and the SuperScript IV PreAmp kit are easy to use, allow reaction setup in a single tube, and are suitable for inputs as low as a single cell. Likewise, both are direct analysis kits that can be used to conduct RT on cell samples without the need for separate RNA isolation or purification steps. In addition to lysis and RT reagents, the SuperScript IV PreAmp kit includes a preamplification step that allows the amplification of obtained cDNA prior to analysis using qPCR or next-generation sequencing (NGS). Table 1 provides the features of each kit that should be considered when planning experiments.

Table 1. Evaluation of the SuperScript IV cDNA kits.

	SuperScript IV CellsDirect cDNA Synthesis Kit	SuperScript IV Single Cell/Low-Input cDNA PreAmp Kit
Kit		
Downstream compatibility	qPCR, ePCR	NGS, qPCR, ePCR
Full length product synthesis	–	+
Second strand synthesis and preamplification	–	+
Protocol time	~35 min	105–140 min (input-dependent)
cDNA synthesis of nonpolyadenylated RNA	+	–
DNA removal	+ (DNase digestion)	+ (mRNA enrichment)
Compatibility with low-quality, degraded samples	+	+*

* Increased degradation levels may lead to loss of sequences representing the 5′ ends of RNA molecules. The impact on downstream qPCR results depends on the position of the targeted region relative to the 5′ end of the RNA molecule. Use of degraded RNA may result in a delayed qPCR signal. Use of degraded RNA for NGS may lead to decreased data quality such as an increased 3′ to 5′ bias and a reduction in sensitivity when compared to sequences from intact RNA.

Growth and preparation of cells used in this study

Table 2 describes the cells that were used in this study.

Table 2. Cells used in this study.

Cells	Description
HEK293T	An immortalized human embryonic kidney cell line. The cells are adhesive and can be grown in 2D and 3D, and are one of the most commonly used cells in biotechnological applications, including gene expression analysis (LGC Standards, Cat. No. CRL-3216).
A549	A cell line derived from human lung adenocarcinoma. The cells are adhesive and can be grown in 2D and 3D, and are primarily used as models for lung cancer studies (ATCC, Cat. No. CCL-185).
Gibco™ Human Spheroid-Qualified Hepatocytes	Primary human hepatocytes. The primary liver cells are obtained from a healthy donor and qualified for growth in 3D (Thermo Fisher Scientific, Cat. No. HMCPSQ).
PBMCs	Primary human peripheral blood mononuclear cells (PBMCs). The cells originate from a fraction of blood cells that consists of lymphocytes and monocytes. The cells do not adhere to the surfaces of growth vessels and do not form 3D structures (Charles River Laboratories, Cat. No. PBC-1000).

HEK293T spheroid growth and preparation

A cell suspension was prepared in prewarmed Gibco™ DMEM growth medium (Cat. No. 11960044) with added Gibco™ Fetal Bovine Serum (FBS) (Cat. No. 10082147) and Gibco™ L-Glutamine (Cat. No. 25030081). A range of 750–1,500 cells was seeded into Thermo Scientific™ Nunc™ MicroWell™ 96-Well Microplates (Cat. No. 260860) that were precoated with 1% agarose to prevent the cells from sticking to the surface of the plate and to promote spheroid formation. The cells were incubated at 37°C with 5% CO_2 for 5 days.

Once spheroids formed, they were transferred with a pipette to 0.2 mL Applied Biosystems™ MicroAmp™ Optical 96-Well Reaction Plates (Cat. No. 4316813) or 1.5 mL tubes and then washed with 100 µL DPBS. DPBS was removed using a pipette, taking care not to disturb the spheroid, until ≤5 µL DPBS remained. The medium was not completely removed to prevent potential spheroid cell loss. Reagents for lysis and cDNA synthesis were added directly to the prepared spheroids.

A549 spheroid growth and preparation

A549 cells were grown in DMEM growth medium with added FBS and L-Glutamine. Approximately 1,500 cells were seeded into each well of a Thermo Scientific™ Nunclon™ Sphera™ 96-Well, U-Shaped-Bottom Microplate (Cat. No. 174925) and incubated at 37°C with 5% CO_2 for 4 days to form spheroids. The spheroids were then carefully transferred to 0.2 mL PCR plates, centrifuged, and collected from the bottom of each well.

Growth medium from each well was carefully removed until ≤5 µL remained. Again, complete medium removal was not performed to prevent potential spheroid cell loss. Reagents for lysis and cDNA synthesis were added directly to the prepared spheroids.

A549 2D cell growth and preparation

A549 cells were grown in DMEM growth medium with added FBS and L-Glutamine. Approximately 1,500 cells were seeded into each well of flat-bottomed 96-well microplates and incubated at 37°C with 5% CO_2 for 4 days. After incubation, growth medium was removed, and cells were immediately used in cDNA synthesis reactions.

Primary hepatocyte spheroid growth and preparation

Gibco Human Spheroid-Qualified Hepatocytes were grown according to the user guide. The resulting spheroids were collected 5 days after seeding and then used in cDNA synthesis reactions.

Primary hepatocyte 2D cell growth and preparation

Human Spheroid-Qualified Hepatocytes were seeded in prewarmed Gibco™ DMEM/F-12, HEPES medium (Cat. No. 11330-032) with added Gibco™ Insulin-Transferrin-Selenium (100X) (Cat. No. 41400-045) and Gibco™ Fetal Bovine Serum (FBS) (Cat. No. 10500064). Before cultivation, the flasks were coated with vitronectin (10 µL vitronectin in 1 mL DPBS). After cultivation, cells were washed with DPBS, dissociated using Gibco™ Trypsin/EDTA Solution (TE) (Cat. No. R001100), suspended in prewarmed growth medium, and counted using a hemocytometer. Approximately 1,500 cells were transferred to 0.2 mL PCR plate wells and immediately used in cDNA synthesis reactions.

PBMCs preparation

The PBMCs were thawed in prewarmed Gibco™ RPMI 1640 Medium (Cat. No. 11875093) with added FBS and 1 mM Thermo Scientific™ sodium pyruvate (Cat. No. J61840.18). Before use, the cells were counted with a hemocytometer and diluted in prewarmed medium (composition identical to thawing medium) to a concentration of ~1,000 cells/µL. Cells were further diluted to ~10 cells/µL using DPBS and immediately used in cDNA synthesis reactions.

cDNA synthesis using the SuperScript IV CellsDirect cDNA Synthesis Kit

Reactions were performed according to the manufacturer's recommendations. Lysis time was 10 min in all cases. Applied Biosystems™ ProFlex™ 3 x 32-well PCR System (Cat. No. 4484073) and 96-well PCR System (Cat. No. 4484075) were used to drive the thermal conditions provided by the kit's user manual.

cDNA synthesis and preamplification using the SuperScript IV Single Cell/Low-Input cDNA PreAmp Kit

Reactions were performed according to the manufacturer's recommendations. All incubation and preamplification steps were performed in ProFlex PCR systems. For all spheroid samples, 11 PCR cycles were used. For PBMC samples, 17 PCR cycles were used. After preamplification, product purification was performed according to the manufacturer's recommendations and the purified product was diluted 3-fold using nuclease-free water to increase the volume for all subsequent reactions.

Purification using TRIzol Reagent and cDNA synthesis using SuperScript IV VILO Master Mix

A pipette was used to transfer HEK293T spheroids to 1.5 mL tubes and A549 spheroids to 0.2 mL tubes for lysis. 1 mL of TRIzol Reagent (Cat. No. 15596018) was used for HEK293T spheroids, and 200 µL were used for A549 cells. Purification was performed according to the manufacturer's recommendations, including optional addition of glycogen. Final resuspension used 30 µL of Thermo Scientific™ nuclease-free water (Cat. No. R0582) for A549 spheroids and 40 µL for HEK293T spheroids. cDNA was synthesized with Invitrogen™ SuperScript™ IV VILO™ Master Mix (Cat. No. 11756500) using 16 µL of purified RNA in a 20 µL reaction. All other reaction conditions were performed according to the provided user manual.

Capillary electrophoresis analysis

The Agilent™ 2100 Bioanalyzer™ Instrument (Agilent, Cat. No. G2939BA) and Agilent™ High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4626) were used to quantify the yields of cDNA products. Electrophoresis was conducted according to the manufacturer's instructions. Quantification was performed by selecting the 300–9,000 bp range.

qPCR analysis

qPCR was performed according to the manufacturer's recommendations using Applied Biosystems™ TaqMan™ Fast Advanced Master Mix (Cat. No. 4444558) and Applied Biosystems™ TaqMan™ Gene Expression Assays (Cat. No. 4331182 and 4448489). In all cases, 2 µL of synthesized nucleic acid product was added as a template into a total qPCR volume of 20 µL. Reactions were performed using Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, 96-well, 0.1 mL (Cat. No. A28568), Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System (Cat. No. 4485688), or Applied Biosystems™ StepOnePlus™ Real-Time PCR System (Cat. No. 4376600). In all cases, a thermal block for 0.1 mL plates and default cycling and data collection settings were used.

Two gene targets were tested in all reactions. The *GAPDH* target was used consistently across all cell types. Apart from *GAPDH*, an additional target was used for each cell type as outlined in Table 3.

Table 3. Specific qPCR targets used for each cell type.

Cell type	qPCR targets
HEK293T	*GAPDH*, *TBP*
A549	*GAPDH*, *CDH1*
Primary hepatocytes (both spheroids and 2D-grown cells)	*GAPDH*, *ALB*
PBMC	*GAPDH*, *CD52*

Results and discussion

Assessing direct cDNA synthesis from 3D cultures of immortalized cell lines

To evaluate the ability of the SuperScript IV PreAmp kit and the SuperScript IV CellsDirect kit to synthesize cDNA directly from 3D cell cultures, both kits were first tested with HEK293T spheroids seeded with varying numbers of cells. For this purpose, spheroids were generated using 750, 1,000, and 1,500 HEK293T cells.

To assess cDNA yields, *GAPDH* and *TBP* gene expression was measured. Similar C_t values were obtained with cDNA samples from both kits, regardless of the number of cells used to seed the spheroids (Figure 1). This result aligns with previously published data indicating that spheroid size is independent of the number of cells seeded, due to a reduced cell division rate and increased apoptosis rate inside internal spheroid layers [6–9].

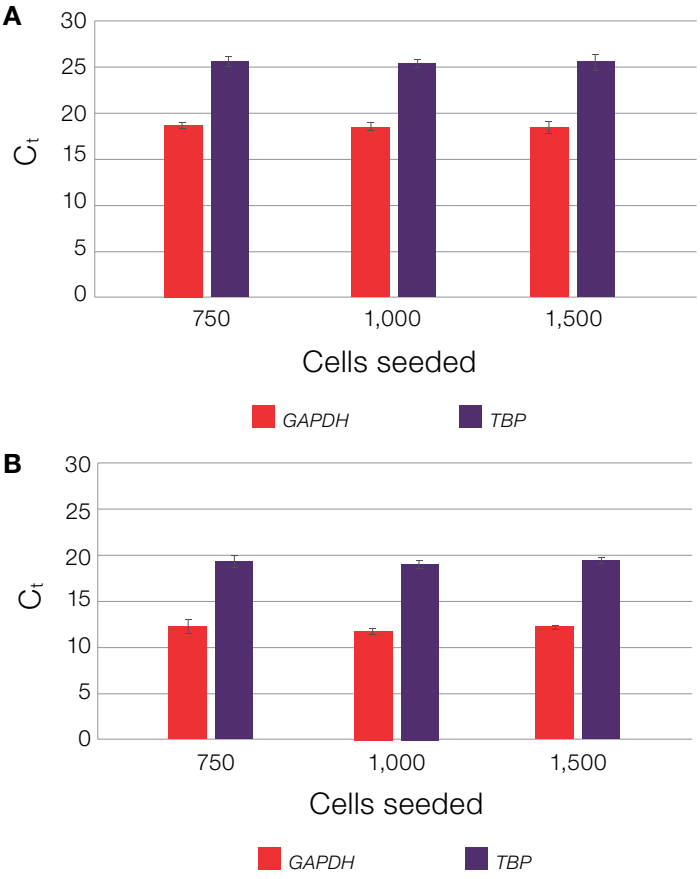

Figure 1. Quantification of cDNA obtained from single HEK293T spheroids grown from 750–1,500 cells. cDNA synthesis was performed using **(A)** the SuperScript IV CellsDirect kit or **(B)** the SuperScript IV PreAmp kit. In all cases, data represent the mean value obtained from multiple (n = 4) spheroid measurements with error bars representing ±1σ (σ = standard deviation).

The difference in C_t values observed between the kits (lower C_t values with the SuperScript IV PreAmp kit) is due to the additional preamplification step, performed only with the SuperScript IV PreAmp kit.

After the preamplification step, the SuperScript IV PreAmp kit yields full-length double-stranded cDNA, and thus its products can be quantified and further verified using capillary electrophoresis (CE). The 300–9,000 bp region of CE data (Figure 2A) was selected for this analysis to exclude peaks caused by leftover preamplification primers (~100 bp) and the upper marker (~10,000 bp). Analysis by CE confirmed that cDNA yields do not depend significantly on the number of cells seeded for spheroid formation (Figure 2B).

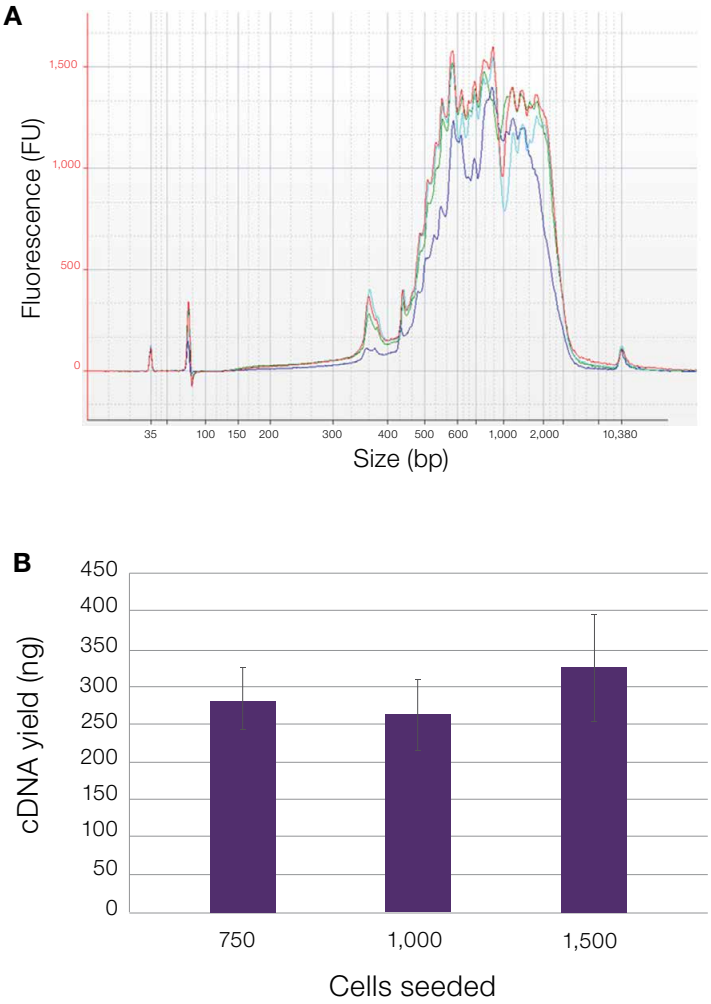

Figure 2. CE analysis of cDNA samples, obtained from HEK293T spheroids, synthesized with the SuperScript IV PreAmp kit. (A) Raw data showing replicate (n = 4) analysis of cDNA from spheroids seeded from 1,500 cells. **(B)** Comparison of cDNA yields, using spheroids from 750–1,500 seeded cells.

In all cases, high cDNA product yields were obtained, confirming that both kits can successfully be used for gene expression analysis from spheroids without RNA isolation steps. Additionally, a preamplification step with the SuperScript IV PreAmp kit enriches mRNA, as CE data (Figure 2A) show the loss of peaks associated with rRNA. This point makes the kit ideal for applications requiring full-length cDNA (e.g., NGS library preparation or endpoint PCR (ePCR) analysis of long transcripts).

Comparison of traditional purification-based methods to direct cDNA synthesis

While traditional cDNA synthesis workflows include lysis and nucleic acid purification prior to RT, it is known that these steps can introduce bias and lead to RNA loss. The direct cDNA synthesis kits evaluated here remove the need for RNA isolation by using robust enzymes that work well in the presence of inhibitors and that therefore can perform cDNA synthesis directly in cell lysate.

To demonstrate the ability of direct cDNA synthesis, HEK293T and A549 spheroid samples were processed using the SuperScript IV CellsDirect kit and compared to those processed with the traditional workflow that uses purification with TRIzol Reagent. The SuperScript IV PreAmp kit was excluded from this analysis because it includes a preamplification step that would increase sensitivity and produce much earlier final C_t values, which would bias the comparison.

With both cell types, the direct cDNA synthesis performed as well, if not better, than the traditional method (Figure 3). C_t values for the direct kits were equal to, or lower than, those for the purification-based workflow approach. These results confirm that the SuperScript IV CellsDirect kit lysed the cells completely and that the SuperScript IV reverse transcriptase was not inhibited by cellular debris or leftover media. Moreover, it was observed that the repeatability was lower for the purification-based method. Higher variance with the purification-based method might result from significantly more hands-on time (Figure 4) and the additional user skill needed (i.e., vision, dexterity, and pipetting skills to repeatably capture a barely visible RNA plaque).

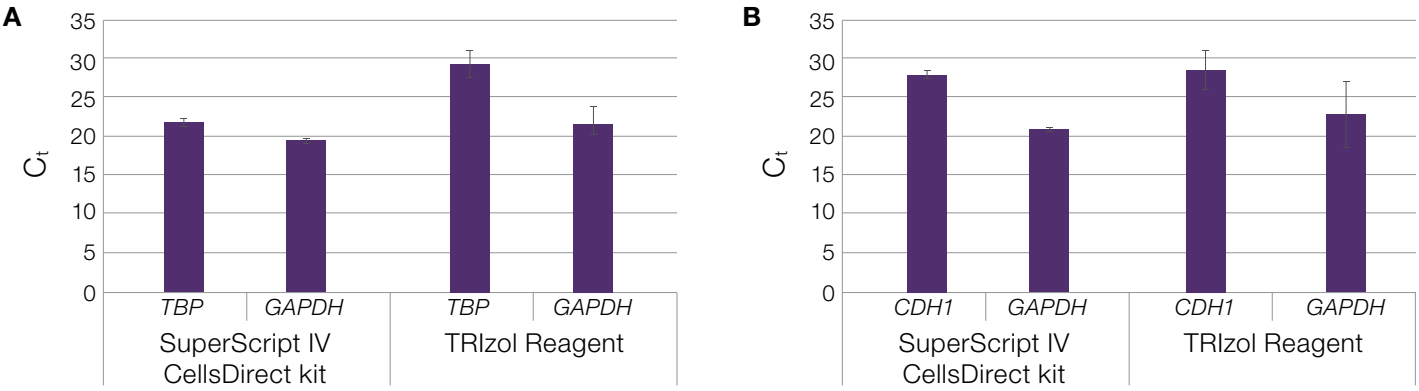

Figure 3. Comparison of C_t values using cDNA samples obtained by direct and traditional RT workflows. With the SuperScript IV CellsDirect kit, intact single spheroids from **(A)** HEK293T cells and **(B)** A549 cells were lysed and used directly in cDNA synthesis. In the traditional workflow, RNA was first purified using TRIzol Reagent and cDNA synthesis was performed using SuperScript IV VILO Master Mix. All data were gathered from single spheroids grown from 1,500 starting cells. C_t values represent the mean value obtained from multiple (n = 5) spheroid samples, with error bars representing ±1σ.

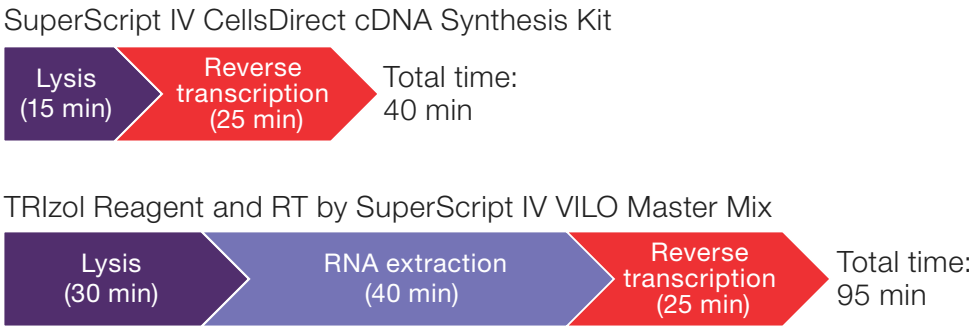

Figure 4. Comparison of the total time required to perform RT using the SuperScript IV CellsDirect kit and an RNA purification-based method. Two products were used to synthesize cDNA with the purification-based method: TRIzol Reagent was used for cell lysis and RNA extraction, and SuperScript IV VILO Master Mix was used for the RT reaction.

Demonstration of cDNA synthesis from primary cell spheroids

Compared to immortalized cell spheroids, primary cell spheroids are significantly more difficult to work with. As an example, it has been shown that similar cell inputs can yield up to 3 times smaller spheroids when using primary cells [6,9]. Futhermore, primary cell spheroids are not as rigid and require more careful handling, both while growing the spheroids and also while preparing for analysis.

Regardless of these additional challenges, both SuperScript IV kits were able to successfully synthesize cDNA from spheroids of primary hepatocytes (Figure 5). As expected, C_t values for primary hepatocyte spheroids were higher when compared to those from spheroids grown from the same number of immortalized cells (see Figure 1). This indicates that analysis of spheroids obtained from primary cells might require more sensitive and efficient RT reagents, particularly for detection of rare targets. For this reason, the SuperScript IV PreAmp kit might be a better option, as it includes the preamplification step, which allows achievement of higher overall yields, earlier C_t values, and more reliable detection of rare transcripts.

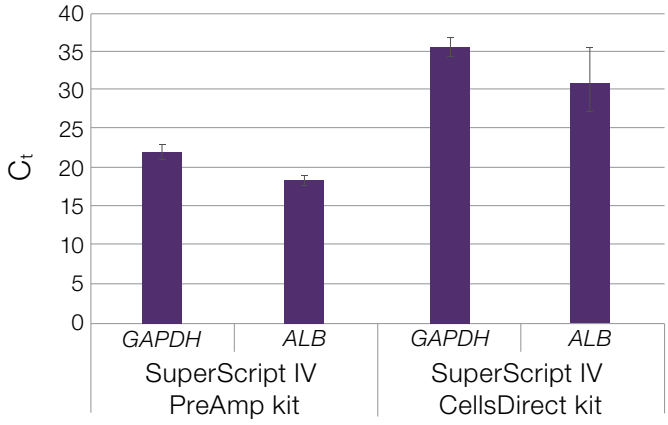

Figure 5. cDNA synthesis from spheroids of primary hepatocyte cells. In all cases, data represent the mean C_t obtained from multiple (n = 6) spheroids with error bars representing ±1σ.

Comparison between spheroids and 2D-cultured cells

Given that the 3D spheroid–derived cells are assumed to be a better model than the 2D-cultured cells, it is important to understand how results from spheroids differ from cells grown in 2D. For such comparison, A549 cells were seeded at a density of 1,500 cells per well and allowed to either form spheroids or grow in a 2D monolayer. After 4 days of growth, the SuperScript IV CellsDirect kit was used to reverse transcribe RNA to cDNA that was then used in qPCR to quantify cDNA yield.

The results shown in Figure 6 indicate that immortalized A549 cells grown in a 2D monolayer produced slightly lower C_t values relative to the same cells grown in 3D spheroids. While not significantly different, these data are consistent across the tested gene targets and support the assumption that spheroids are expected to exhibit a reduced cell proliferation rate and have increased rates of cell death [6–8].

A similar comparison of 3D vs. 2D cell samples was also conducted with primary hepatocytes. Due to the smaller size of primary cell–grown spheroids, it was expected that far lower cDNA yields would be obtained as compared to cells grown

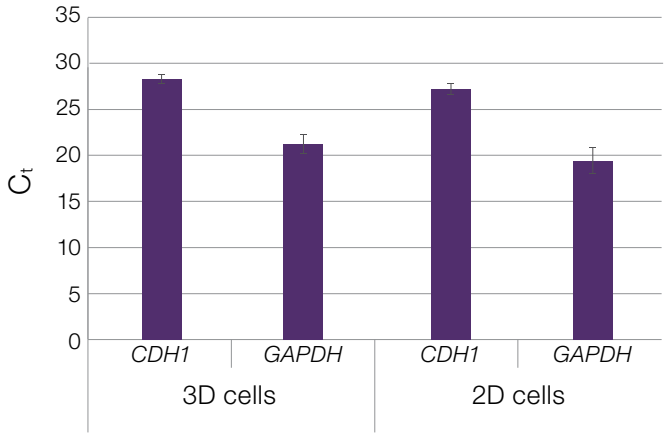

Figure 6. Comparison of cDNA yields produced by the SuperScript IV CellsDirect kit using immortalized A549 cells grown in a 2D monolayer and 3D spheroids. In all cases, data represent the mean C_t for *CDH1* and *GAPDH* targets, obtained from multiple (n = 6) measurements with error bars representing ±1σ.

in 2D culture. Primary hepatocytes, grown in 2D culture, were diluted in growth medium to a concentration of 1,500 cells/µL. A 1 µL aliquot of this suspension was directly used in cDNA synthesis reactions with the SuperScript IV CellsDirect and SuperScript PreAmp kits. Results for these cells grown in 2D culture were then compared to results for 3D spheroid–derived hepatocyte cells (Figure 7). C_t values obtained from spheroids were up to 5 cycles higher when compared to 2D culture–derived cells, indicating that cell number is decreasing during spheroid formation [7,8]. These results confirm that cells grown in 3D environments require more sensitive analysis methods for accurate gene expression analysis. Thus, the SuperScript IV PreAmp kit may be a better option for the detection of rare transcripts from such inputs, as the additional preamplification step allows for higher yields and earlier C_t values.

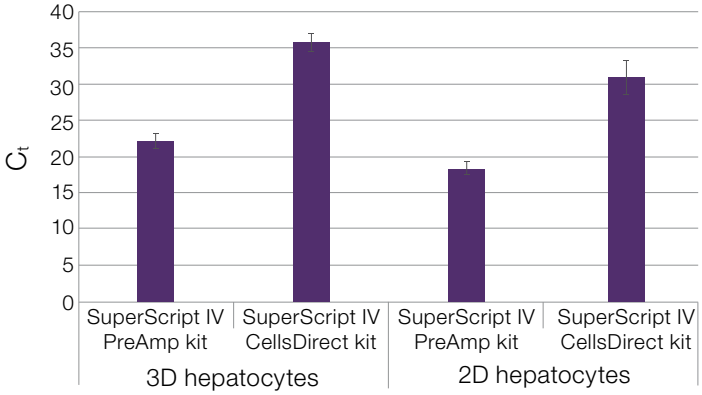

Figure 7. Comparison of cDNA yields from both SuperScript kits based on input from primary hepatocytes that were grown in either 2D or 3D. Differences in C_t values between the two kits are caused by the additional preamplification step provided by the SuperScript PreAmp kit. In all cases, data represent the mean value obtained for the *GAPDH* target from multiple (n = 6) measurements with error bars representing ±1σ.

Reverse transcription of other, nonspheroid-derived primary cell RNA

While the above results are promising, they do not yet demonstrate the use of the kits with noncultured primary cells. For this, both SuperScript IV kits were tested with a small number (n ≈ 10) of PBMCs, followed by quantification of reaction products using qPCR.

Data indicate (Figure 8) that both kits were sensitive enough to detect the target of interest from as few as 10 of these primary cells. Although both kits were successful in providing sufficient cDNA yield to generate a qPCR signal, C_t values obtained using the SuperScript IV CellsDirect kit were near detection limits, while those obtained using the SuperScript IV PreAmp kit were significantly lower. This result indicates that the preamplification step can be valuable when working with minimal sample inputs and low-abundance targets.

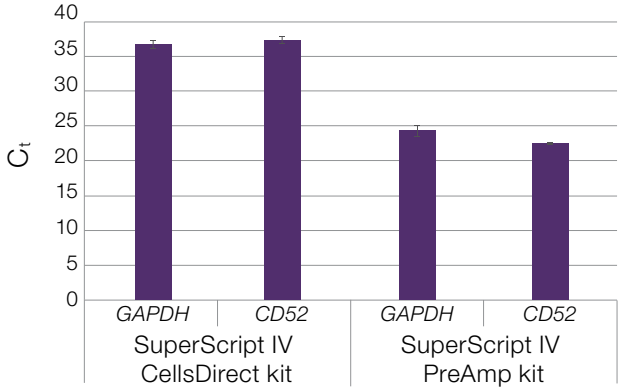

Figure 8. cDNA synthesis from as few as 10 primary PBMCs. In all cases the *GAPDH* and *CD52* gene targets were detected. However, the SuperScript IV PreAmp kit produced lower C_t values, indicating it is ideal for low copy number, rare-transcript applications. In all cases, data represent the mean value from multiple (n = 5) measurements with error bars representing ±1σ.

Conclusion

The need to derive meaningful results from low-input and difficult samples can only be expected to continue over time. This necessitates that reagent and instrument providers continue to advance their products to meet the needs of the scientific community. Without any protocol modifications, the SuperScript IV CellsDirect cDNA Synthesis Kit and SuperScript IV Single Cell/Low-Input cDNA PreAmp Kit have been shown to successfully support transcriptomic and gene expression analysis workflows using low-input cell numbers and a variety of difficult samples, including primary cells and 3D cultures. The two kits were shown to be highly sensitive, which should be a priority given that 3D cultures, while they may better mimic *in vivo* conditions, can be self-limiting and provide less RNA for analysis. Furthermore, both kits enable direct reactions that provide superior results compared to purification-based methods while also saving valuable time.

While both kits offer clear advantages over traditional methods, selection of the most appropriate kit depends on end-user requirements and planned downstream analysis methods. The SuperScript IV CellsDirect cDNA Synthesis Kit is suitable in most cases that require quantification or gene expression analysis using qPCR. It enables fast cDNA synthesis and is fully compatible with degraded RNA. The SuperScript IV Single Cell/Low-Input cDNA PreAmp Kit, which provides global preamplification of full-length cDNA, is a better option in more specific cases. These cases include, but are not limited to, synthesis of NGS library preparation–compatible double-stranded cDNA, synthesis of full-length cDNA and preamplification for downstream analyses that require large nucleic acid inputs, and the use of extremely low-input amounts, especially if detection of rare transcripts is required.

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Ordering information

Product	Quantity	Cat. No.
SuperScript IV CellsDirect cDNA Synthesis Kit	50 reactions	11750150
	500 reactions	11750350
SuperScript IV Single Cell/Low-Input cDNA PreAmp Kit	48 reactions	11752048
	96 reactions	11752096
	192 reactions	11752192
	384 reactions	11752384
	480 reactions	11752480

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