# Direct amplification of cell culture samples


## Abstract

Biological extractions are essential for the collection of nucleic acids and removal of PCR inhibitors before quantitative PCR (qPCR) reactions are performed. In this technical note we will explore using IDT's PrimeTime™ One-Step 4X Broad-Range Master Mix as an alternative to other costlier and more time-consuming extraction approaches. Here, we show how extraction-free amplification can efficiently amplify cell culture samples when compared to controls. Further, the data show similar performance across all four markers queried.


## Introduction

Animal cell culture has become a mainstay laboratory technique, essential for the study of molecular, biochemical, and physiological processes. Cell culture encourages rapid expansion of cells and even clonal expansion of a single cell. This technique provides faster experiment turnaround time from conception to data analysis and serves as an attractive alternative to tissue samples. While cell culture work can be challenging—requiring specific growth conditions, avoiding contamination, and extracting protein or nucleic acids for analysis—novel techniques and molecular biology reagents are bringing cell culture techniques within reach for most molecular laboratories.

Complimentary to cell culture technique, qPCR is gaining in popularity within the testing community as the go-to method for these tests. qPCR does offer distinct advantages over NGS and cell culture, including a reduction in cost and turnaround time. Additionally, qPCR has increased accuracy in discriminating between pathogens and it offers the beneficial option to query multiple targets in a single reaction through multiplexing assays.

Traditionally, extra steps were involved extracting nucleic acids from the sample of interest. This could slow down the time from sample collection to results by 1–2 hours. With the introduction of IDT's PrimeTime One-Step 4x Broad-Range Master Mix, researchers are changing their approach. Because the Broad-Range Master Mix allows you to skip the timely extraction step, researchers can go directly from sample collection to amplification without a loss in performance. The basic outline for this testing workflow is shown in Figure 1. Simply eliminating the extraction step can save researchers $1–2/sample, per extraction. Here, we will delve into direct amplification of cell culture samples and examine how the data compares to normalized controls.

# Materials and Methods

## Quantitative PCR

We performed qPCR using PrimeTime One-Step 4X Broad-Range Master Mix without direct amplification enhancer solution (IDT) and with Custom DNA Oligos for primers (500 nM final concentration) (IDT) and PrimeTime probes (250 nM final concentration) (IDT). Collection of the cell culture samples occurred by one of four collection methods: 1) 2 mL of cell lysis buffer (10 mM Tris-HCl pH 7.4, 0.25% IGEPAL CA-630, 150 mM NaCl) followed by 5 min incubation, 2) scraped in 2 mL of DMEM, 3) scraped in 2 mL of 1X PBS, or 4) trypsinized using 100 µL of Trypsin followed by the addition of 1900 µL of DMEM. The assay master mix and cell culture samples were mixed at a 10:1 ratio for a final volume of 20 µL. The cell culture samples were compared to human normalized panel control normalized to 300,000 copies in a TE + tRNA background. The reaction proceeded on a qPCR (QuantStudio7 Flex) using the following method: 50°C for 15 min, 95 C for 30 sec, followed by 45 cycles at 95°C for 5 sec, then 60°C for 45 sec.

# Results

Normal laboratory workflow requires trypsinization of cells to dissociate cells from the support in which they are being cultured, followed by extraction to isolate the DNA or RNA from within. To establish a baseline for direct PCR amplification, trypsinized cell samples were directly added to a qPCR reaction and assessed for their ability to amplify one of four mRNA targets. Direct amplification of the trypsinized samples was similar to the amplification of the normalized control for all four markers tested (Figure 2A). While trypsin proved to be an effective method of harvesting cells for direct amplification, additional experiments were performed to explore other collection methods and assess their performance within a direct amplification protocol looking at the same four markers (Figure 2B). Similar to cells collected by a trypsin protocol, cell lysis buffer and scraping of the plates with DMEM or PBS performed similarly compared to the human normalized control. While there is slight deviation in the amplification of the four sample types compared to the human normalized control, this may be the result of variations in the input nucleic acid compared to the quantified control.

# Conclusion

The results showed that the use of IDT's PrimeTime One-Step 4X Broad-Range Master Mix is an effective solution for direct amplification of cell culture samples collected by trypsinization. Furthermore, lysis buffer and scraping of plates also proved to be suitable methods for collection of samples for direct qPCR amplification. While direct amplification performed as well as normalized controls, additional experiments are needed to compare the outcomes of direct amplification to amplification following traditional extraction protocols. The implementation of direct amplification results in faster times from sample collection to analysis of data and can reduce overall experimental costs by removing costly extraction steps.

## Direct amplification of mRNA targets under differing collection conditions

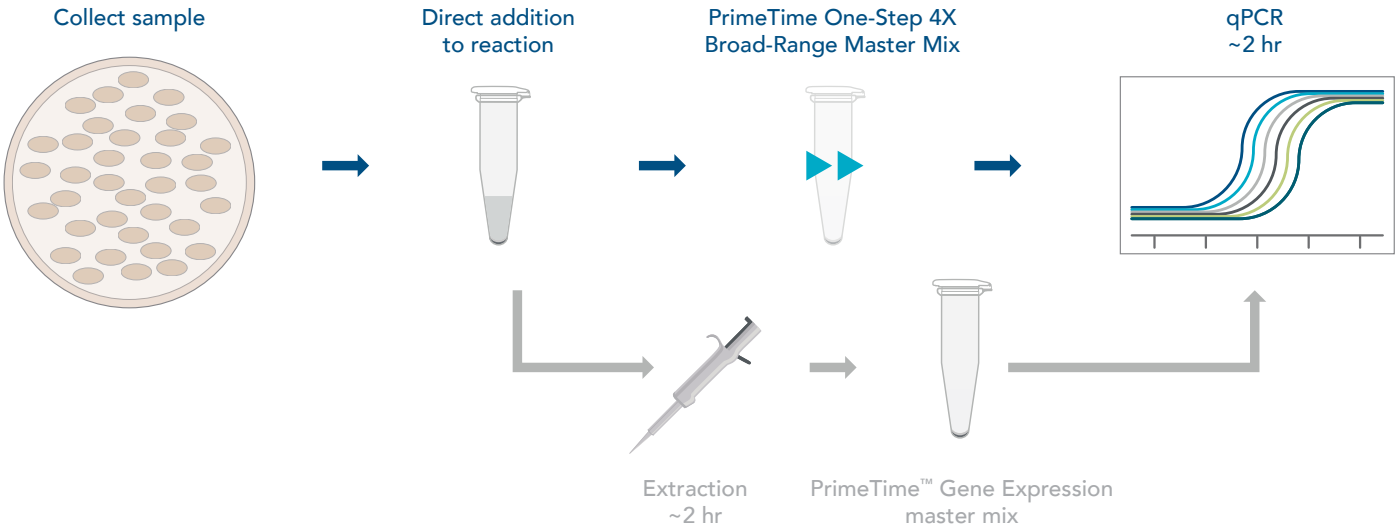


**Figure 1. Basic workflow of direct amplification of cell culture samples.** Leveraging the PrimeTime One-Step 4X Broad-Range Master Mix gives the researcher the opportunity to shorten the workflow by skipping the lengthy and costly extraction step (saving ~2 hours and ~$1-2/sample on extraction).

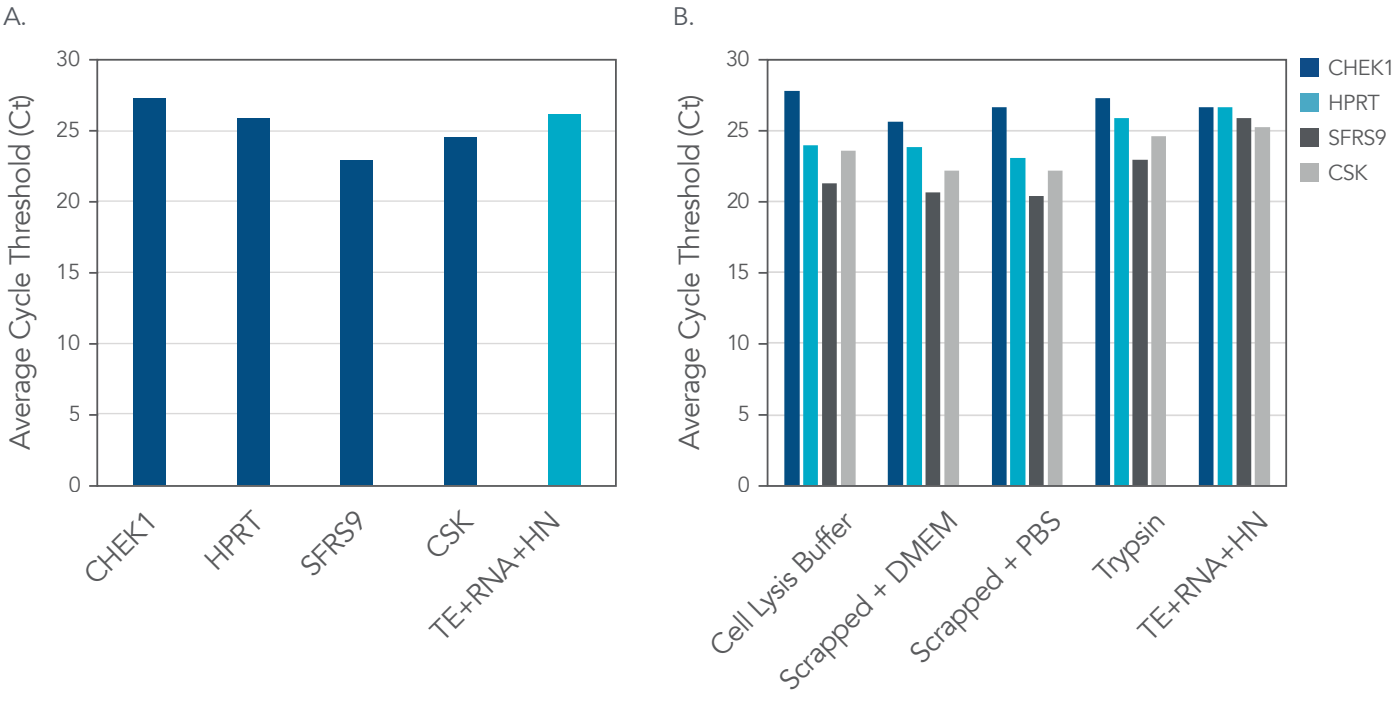


**Figure 2. Amplification data for direct amplification of cell culture targets under different collection conditions.** (**A**) Amplification of four different mRNA targets using cells collected by trypsinization using PrimeTime One-Step 4X Broad-Range Master Mix without the included direct amplification enhancer, (**B**) Amplification of four different mRNA targets using cells collected by Cell Lysis Buffer, DMEM + scraping, PBS + scraping, or by trypsinization using PrimeTime One-Step 4X Broad-Range Master Mix without the included direct amplification enhancer.

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Doc ID: RUO23-2044_001 07/23