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Applied Biosystems™ TaqMan™ Gene Expression Assays for droplet-based digital PCR systems

Gene expression

Summary

Applied Biosystems™ TaqMan™ Gene Expression Assays support precise and reproducible quantification across different digital PCR (dPCR) platforms. In this study, predesigned TaqMan™ assays were evaluated on both a Microfluidic Array Plate (MAP) platform and a droplet-based dPCR platform for relative quantification and detection of small fold changes. The evaluated assays demonstrated consistent analytical performance and were successfully transferred across both platforms without requiring assay redesign. Endogenous control assays delivered reliable results across different dye configurations, showed consistent accurate quantification over a dilution series, and produced clear cluster separation. Representative duplex assays resolved 10% incremental expression changes in contrived research samples, indicating reliable results across workflows.

Introduction

Gene expression analysis is a foundational tool in biological and translational research. Researchers rely on accurate measurement of mRNA levels to characterize cellular pathways, identify disease-associated signatures, and assess potential therapeutic targets. Quantitative real-time PCR (qPCR) has long served as a trusted method for gene expression studies because it offers analytical sensitivity, a broad dynamic range, and straightforward implementation [1]. However, as research questions become more refined, the demand for higher resolution has grown, including the need for precise measurements of low-abundance transcripts and subtle fold changes [2].

Digital PCR (dPCR) enables precise nucleic acid quantification with high analytical sensitivity by dividing a PCR reaction into thousands of individual microreactions, into which DNA or RNA molecules are randomly distributed. Following amplification, each microreaction is classified as positive or negative based on fluorescence detection. The proportion of positive reactions is analyzed using Poisson statistics to determine the absolute number of target molecules without the need for a standard curve. By isolating targets into independent microreactions, dPCR minimizes template competition and reduces the impact of PCR inhibitors, resulting in greater analytical sensitivity, precision, and reproducibility than qPCR. These advantages make dPCR particularly well suited for the quantification of low-abundance targets, detection of rare transcripts, and resolution of subtle differences in gene expression [3,4].

In dPCR, reaction compartmentalization is primarily achieved through two approaches: physical separation into microchambers or generation of water-in-oil emulsion droplets. In microchamber-based systems, such as the MAP (microfluidic-array-plate) system, samples are compartmentalized into thousands of microchambers within a sealed plate, offering a streamlined workflow that is automation-friendly and minimizes pipetting errors, enabling fast, reliable results.

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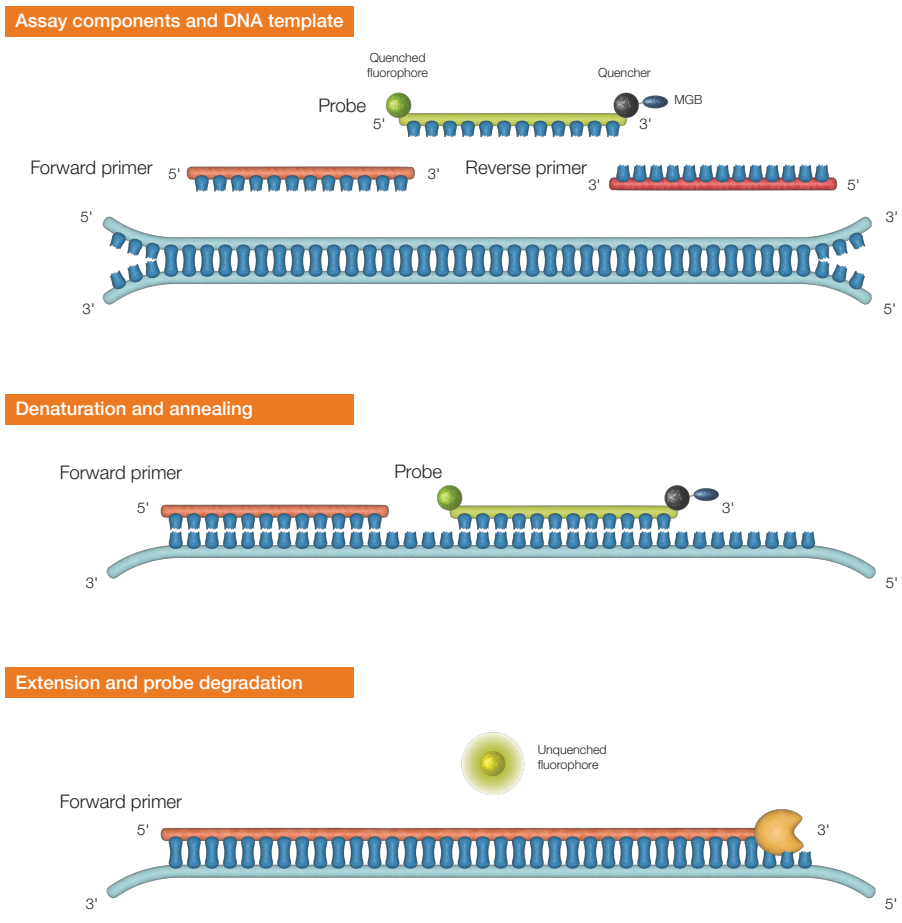
In comparison, the droplet-based dPCR (ddPCR) system is a specific dPCR approach that compartmentalizes reactions into thousands of nanoliter-sized droplets using a water-in-oil emulsion system. This workflow requires a dedicated droplet-generation step prior to thermal cycling, followed by droplet reading and analysis to determine the number of positive and negative droplets for quantification.

As dPCR becomes increasingly adopted for gene expression analysis, researchers must balance analytical performance with practical considerations such as assay availability, multiplexing requirements, and cross-platform compatibility. Many laboratories operate multiple platforms or transition between qPCR and dPCR during a project, while others require multiplex configurations that accommodate specific dye channels supported by their instruments [5]. These realities create a need for a large, reliable portfolio of predesigned assays that can be deployed across platforms without needing to redesign or re-optimize.

Applied Biosystems™ TaqMan™ Gene Expression Assays address this need by offering an extensive collection of predesigned assays covering a broad range of gene targets (Figure 1). Assays are available with multiple fluorophore options, enabling flexible dye assignment to fit instrument capabilities and multiplex design requirements. Since the same assay designs can be used in both qPCR and dPCR workflows, researchers can maintain continuity across platforms while adapting to evolving experimental needs.



Why choose predesigned Applied Biosystems TaqMan gene expression assays for dPCR?

Applied Biosystems TaqMan assays are designed using our sophisticated design pipeline. Each inventoried, made-to-order, and custom assay undergoes the following process:

- Target selection using NCBI databases
- Preprocessing, including mapping to genome, masking of SNPs, repeats, and discrepancies, and identification of exon-exon junctions
- Assays designed with balanced T_m for universal thermal cycling, minimization of secondary structures, optimization of GC content and amplicon size, and elimination of primer dimers
- *In silico* quality control scoring for target and genome specificity
- Stringent assay formulation QC and identity confirmation by mass spectrometry

More than 2.8 million catalog assays for over 30 species, backed by a performance guarantee*

Figure 1. Overview of Applied Biosystems TaqMan assays. TaqMan assays consist of a forward primer, a reverse primer, and a probe labeled with a fluorophore at the 5' end and a quencher and minor groove binder (MGB) at the 3' end. During the annealing step, the primers and probe anneal to the denatured template. During the extension step, the 5' to 3' exonuclease activity of Taq polymerase hydrolyzes the probe, separating the fluorophore from the quencher and permitting fluorescence.

*Terms and conditions apply. For complete details, visit **thermofisher.com/taqmanguarantee**.

In this technical note, we evaluated TaqMan gene expression assay performance across two digital PCR platforms: a MAP-based system and a droplet-based system. Endogenous control assays were tested in different dye configurations to demonstrate consistent quantification, regardless of fluorophore selection. This flexibility allows researchers to select dye formats that best fit their multiplex design needs without compromising accuracy. In addition, two duplex lung cancer research assays were used to assess multiplex discrimination and the resolution of small fold changes between healthy and diseased samples. Across both technologies, results demonstrate reproducible quantification and clear sample differentiation, supporting the use of predesigned TaqMan gene expression assays on a variety of dPCR platforms.

Materials and methods

Three endogenous control targets were evaluated using the following TaqMan gene expression assays: *HPRT1* (Assay ID: Hs99999909_m1), *RPLP0* (Assay ID: Hs99999902_m1), and *POLR2A* (Assay ID: Hs00172187_m1). The three assays, which contained Applied Biosystems™ FAM™ and VIC™ dyes, were formulated into duplexes. cDNA for endogenous control testing was generated from Invitrogen™ Universal Human Reference RNA (UHR) for Invitrogen™ QuantiGene™ Assays using a standard reverse transcription workflow according to the manufacturer's instructions. A serial dilution series of the resulting cDNA was prepared to evaluate assay performance across a broad dynamic range of expression levels, reflecting the variability in transcript abundance commonly encountered in gene expression studies.

For the duplex lung cancer research assays, contrived samples were prepared using cDNA as the background matrix. cDNA was generated from total human lung RNA control (catalog number AM7968) using a standard reverse transcription workflow according to the manufacturer's instructions. Quantified synthetic DNA templates corresponding to *CD274* and *SOX2* was spiked into lung cDNA to generate synthetic expression profiles representing healthy and diseased subsets with small incremental fold differences. Two duplex assays were formulated to detect *CD274* (Assay ID: Hs00204257_m1, FAM dye, lung duplex 1), *SOX2* (Assay ID: Hs00415716_m1, FAM dye, lung duplex 2), and *CIAO1* (Assay ID: Hs00191307_m1, VIC dye, lung duplex 1 and 2). *CIAO1* was included in both lung duplex 1 and 2 as the reference gene based on published evidence supporting stable expression in lung tissue [6]. Catalog assays are available in multiple fluorophore configurations and may be ordered with alternative dyes through the Applied Biosystems™ Specialty TaqMan™ Assay and Oligo Service to accommodate multiplex design requirements (see Table 1).

dPCR experiments were performed on a MAP-based system and a droplet-based system using the manufacturer-recommended master mixes, thermal cycling protocols, and analysis settings for each platform. Absolute target concentrations were calculated using platform-specific Poisson analysis software.

Results

Consistent quantification across dyes

To evaluate whether fluorophore selection impacts quantitative performance, three endogenous control assays—*HPRT1, POLR2A,* and *RPLP0*—were tested in a duplex format using FAM- and VIC-labeled probes on the two dPCR platforms. As shown in Figure 2, serially diluted cDNA produced normalized quantification values that were consistent with the expected values for the two

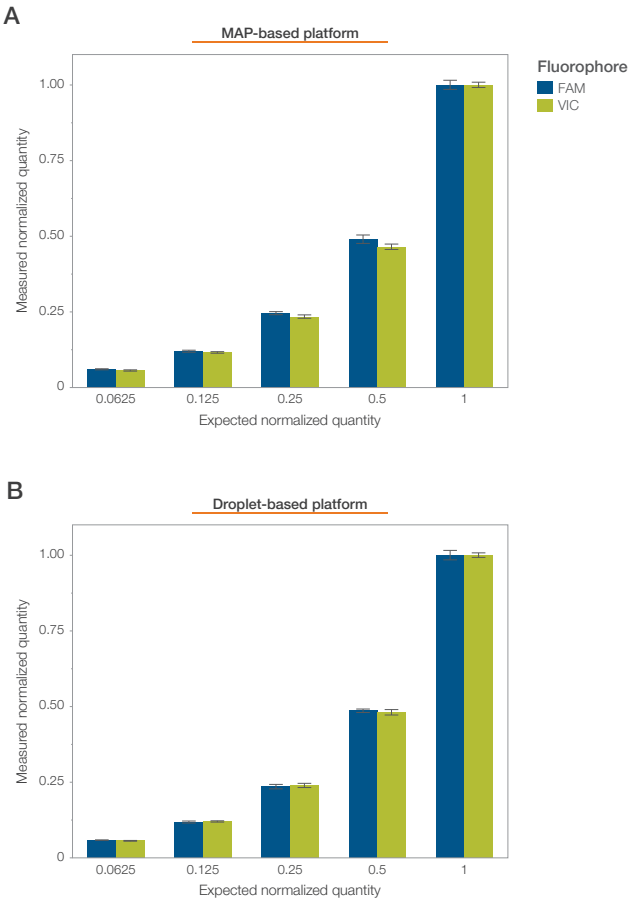


Figure 2. Consistent *RPLP0* quantification across fluorophores and platforms. Quantities were calculated by normalizing each dilution to the mean concentration of the undiluted sample for each dye. Two-fold serial dilutions were prepared from the highest input sample. The x-axis represents expected value based on dilution factor, and the y-axis shows mean normalized values. Error bars represent standard deviation of four replicates per dilution. **(A)** Representative *RPLP0* quantification for FAM and VIC on the MAP-based platform. **(B)** Representative *RPLP0* quantification for FAM and VIC on the droplet-based platform.

fluorophores and on both platforms, demonstrating a proportional response independent of fluorophore assignment in the duplex format. Distinct positive and negative populations and clear cluster separation were observed for each target and dye in duplex (representative data are shown in Figure 3).

Detection of common lung cancer research targets

To evaluate performance in a representative gene expression application, two duplex lung cancer research assays were formulated. Lung duplex 1 consisted of *CD274* with *CIAO1* as the reference gene while lung duplex 2 consisted of *SOX2* with *CIAO1* as the reference gene. The duplex assays were assessed using contrived samples formulated to mimic healthy and diseased profiles with small fold changes in expression. Relative quantification (RQ) values were calculated for each sample by normalizing *CD274* and *SOX2* concentrations to the endogenous control gene *CIAO1* within the same reaction, then expressing that ratio relative to the reference control sample.

As shown in Figure 4, 10% incremental increases in normalized *CD274* expression were observed on both platforms across the contrived sample set and reflected the small fold changes in input RNA. Consistent *CIAO1* expression was observed across all contrived samples on both systems (data not shown). Notably, this stable endogenous control signal was maintained while target biomarker concentrations were titrated, highlighting the capability of dPCR to simultaneously quantify a stable reference gene alongside targets with small fold changes within a single reaction.

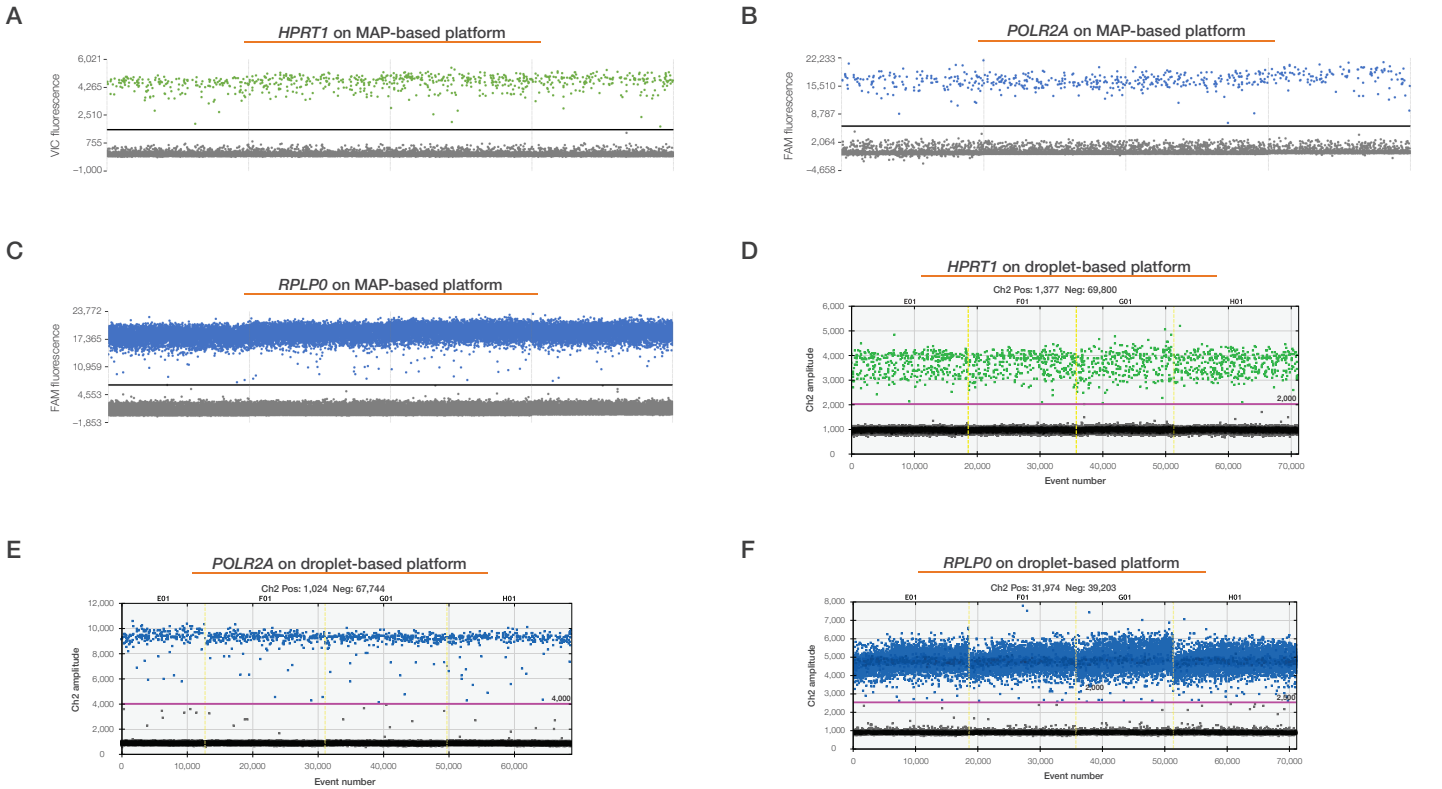


Figure 3. Representative one-dimensional (1D) fluorescence amplitude plots demonstrating multiplex signal separation. *HPRT1, POLR2A*, and *RPLP0* were combined in a duplex reaction and detected using FAM and VIC dyes. Manual fluorescence thresholds were applied for each target across samples (solid horizontal line). Four replicates of dilution 0.5 are shown. RFU, relative fluorescence units. Representative plots of the genes in either FAM or VIC dye are shown. (A-C) Representative plots from the MAP-based platform. (D-F) Representative plots from the droplet-based platform. Gray and black dots indicate negative microreactions. (A, D) *HPRT1* (VIC). Green dots indicate positive microreactions. (B, E) *POLR2A* (FAM). Blue dots indicate positive microreactions. (C, F) *RPLP0* (FAM). Blue dots indicate positive microreactions.

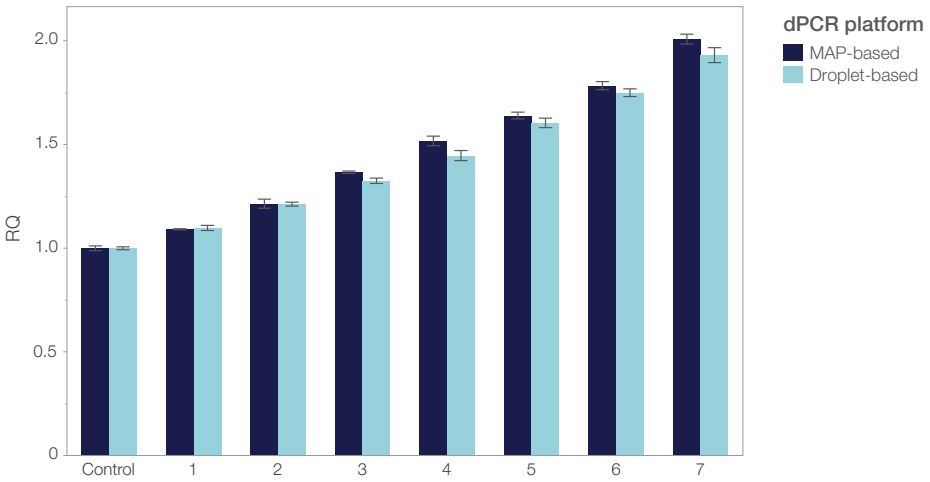


Figure 4. Relative quantification of *CD274* in contrived lung cancer samples on two dPCR platforms. Samples containing 10% incremental increases in *CD274* relative to the control sample were tested on both the MAP-based and droplet-based dPCR platforms. Relative quantification (RQ) values were calculated by normalizing *CD274* concentration to *CIAO1* within each reaction, then expressing the normalized value relative to the reference control (Control). Bars represent mean RQ values, and error bars indicate the standard deviation for four replicates.

Conclusion

In this technical note, TaqMan gene expression assays demonstrated consistent performance across two independent digital PCR technologies. The assays maintained consistent quantification across dyes, supporting flexible fluorophore assignment to accommodate multiplexing. Clear separation between positive and negative microreactions was achieved. In the duplex lung-cancer research assays, small incremental biomarker expression changes were resolved against a background of strongly positive endogenous control expression in both systems, illustrating that a stable reference gene and diverse biomarker expression patterns can be quantified simultaneously in a duplex format.

Together, these results support the use of TaqMan Gene Expression Assays on the digital PCR platforms tested. With an extensive portfolio of predesigned TaqMan assays, multiplexing support tools, and access to specialty oligo services for alternative dye configurations, researchers can design and implement multiplex gene expression panels tailored to their specific instrument requirements and experimental needs.

References

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Table 1. Ordering information

Description	Link
TaqMan Gene Expression Assays	thermofisher.com/taqmangeneexpression
Custom assays	thermofisher.com/order/custom-assay-design-tool
Catalog assays with custom dyes	thermofisher.com/taqman-custom-assay-oligo
Multiplexing support	thermofisher.com/us/en/home/global/forms/life-science/assay-support.html

Learn more at **thermofisher.com/taqman-dPCR**