Digital PCR

Fast and sensitive absolute quantitation of RNA targets with the Absolute Q 1-Step RT-dPCR Master Mix (4X)

Features include:

- **Fast time-to-results**—5 minutes of hands-on time, and results in less than 2 hours
- **Simple workflow**—set up the reaction like a typical 1-step RT-qPCR, then load and run the plate in a single instrument
- **High sensitivity**—ability to detect <1 copy/µL of a specific target in a 4-plex reaction
- **Optimized for multiplexing**—detect up to 4 targets in a single reaction
- **Reproducibility**—highly reproducible results between technical replicates; consistently generates >20,000 analyzable microchambers

The Applied Biosystems™ Absolute Q™ 1-Step RT-dPCR Master Mix (4X) is optimized for use with the Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System in a simple workflow with minimal processing steps. The 4X formulation enables analysis of higher sample volumes and delivers precise, accurate quantification of RNA targets without using a standard curve.

Simple and efficient workflow

The QuantStudio Absolute Q Digital PCR (dPCR) System simplifies the entire dPCR workflow. Prepare the dPCR reaction mix as you would for qPCR, pipette the reaction mix and isolation buffer into the microfluidic array plate (MAP), and place the plate in the QuantStudio Absolute Q dPCR System. Compartmentalization, thermal cycling, and data acquisition for up to 4 targets occur in a single instrument on a single dPCR plate (Figure 1). This simple workflow requires only 5 minutes of hands-on time to set up, and the dPCR data are ready to view and analyze in less than 2 hours.

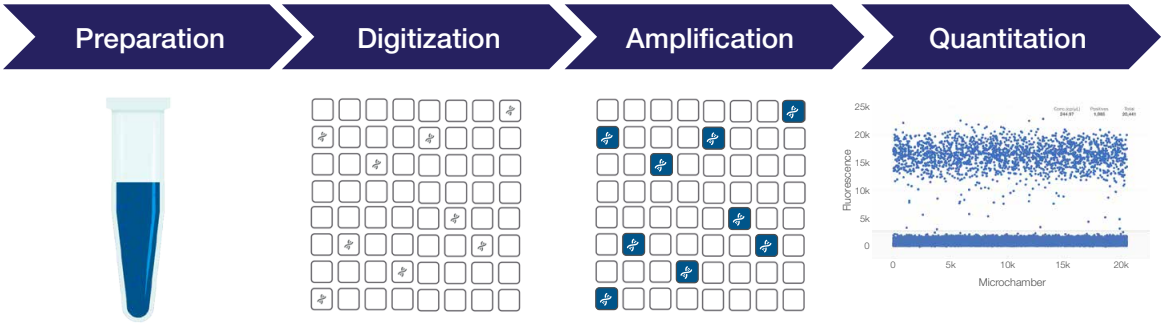

Figure 1. Efficient dPCR workflow. All steps of dPCR are completed on a single instrument in under 2 hours, with less than 5 minutes of hands-on time.

Robust multiplexing

Simultaneous detection and quantification of multiple RNA targets is important for many applications. Multiplexing targets in dPCR reactions facilitates absolute quantification of more data points per sample volume tested, as well as enabling higher-precision quantification for rare or low-concentration targets within a heterogeneous sample. The Absolute Q 1-Step RT-dPCR Master Mix was designed for optimal dPCR performance, enabling highly sensitive detection of rare or low-concentration targets down to or below 1 copy/µL in assays containing up to 4 multiplexed targets.

To demonstrate the capability of the Absolute Q 1-Step RT-dPCR Master Mix for highly sensitive detection of rare targets, a 4-plex assay was designed to detect SARS-CoV-2 targets, as well as Applied Biosystems™ VetMAX™ Xeno™ Internal Positive Control (IPC), in wastewater. The VetMAX Xeno IPC was spiked into a SARS-CoV-2 wastewater control sample at a final concentration of ≤1 copy/µL. Using the Absolute Q 1-Step RT-dPCR Master Mix in a 4-plex assay, both the control mixture and a no-template control (NTC) were tested using the QuantStudio Absolute Q dPCR System. Figure 2 highlights the results of the dPCR experiment, showing detection of the VetMAX Xeno IPC at ≤1 copy/µL in the presence of three SARS-CoV-2 targets at 95% confidence relative to the NTC.

High sensitivity

dPCR facilitates high-precision detection and quantification of targets that are at low concentrations. To showcase the low limits of detection, the Absolute Q 1-Step RT-dPCR Master Mix was used with a 3-plex wastewater assay (N1 and N2 SARS-CoV-2 targets and pepper mild mottle virus (PMMoV) DNA control) to detect rare SARS-CoV-2 RNA targets present at 1 copy/µL. Control SARS-CoV-2 material and PMMoV were diluted to 1 copy/µL and run alongside a no-template control (NTC) in quadruplicate. For the three RNA targets, the mean concentrations detected in the control conditions were significantly different compared to the NTC conditions using the Student's t-test ($p = 0.0002$ and 0.0004 for N1 and N2, respectively). These results indicate that targets as low as 1 copy/µL were detected and statistically differentiated from the NTC conditions (Figure 3).

High precision and accuracy

Without relying on standard curves, dPCR has higher precision to detect minute concentration differences between samples. To demonstrate high precision and accuracy, the Absolute Q 1-Step RT-dPCR Master Mix was used to quantify the SARS-CoV-2 N1 and N2 RNA targets at three concentrations differing by 10%. Figure 4 highlights the quantitative results, with each concentration point distinguishable with 95% confidence.

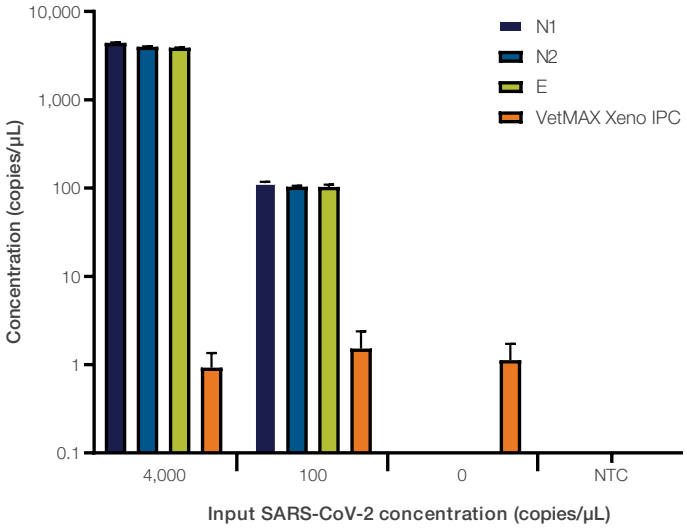

Figure 2. Sensitive detection of low-concentration targets of <1 copy/µL in a 4-plex reaction. A dilution series of SARS-CoV-2 control material containing the N1, N2, and E gene targets was prepared at 4,000, 100, and 0 copies/µL. VetMAX Xeno Internal Positive Control (IPC) was spiked into each condition at a concentration of 1 copy/µL. All three targets were measured using a 4-plex dPCR assay.

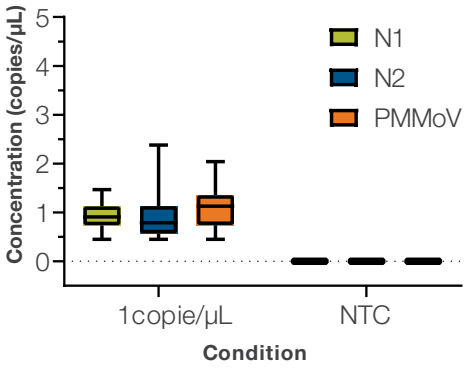

Figure 3. Sensitive detection of low-concentration targets down to 1 copy/µL for three RNA targets, compared to a no-template control (NTC). Box and whisker plots show the minimum, maximum, and median concentration for each target. Mean concentration and standard deviation were 0.93 ± 0.29 for N1, 0.97 ± 0.56 for N2, and 1.13 ± 0.49 for PMMoV. All means were statistically significant using the Student's t-test ($p < 0.01$), compared to the NTCs with 0 copies/µL.

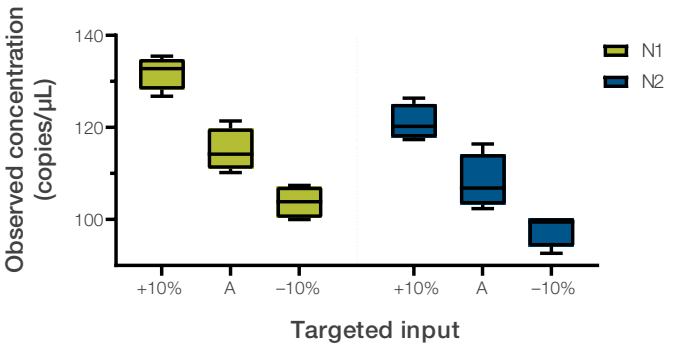

Figure 4. Discrimination of 10% differences in RNA target concentration with 95% confidence.

High reproducibility for single-plex and multiplex reactions

To demonstrate reproducibility of dPCR quantification, a series of 4 RNA targets were measured using 1-plex dPCR reactions as well as one 4-plex reaction. Quantification of each target was consistent between the single-plex and multiplex dPCR reactions (Figure 5).

Optimized for maximum reagent efficiency

The Absolute Q 1-Step RT-dPCR Master Mix was designed for optimal performance on Applied Biosystems™ QuantStudio™ MAP16 digital PCR plates. To measure the consistency of reagent digitization, the total number of microchambers accepted for analysis was measured across 32 independent dPCR reactions. By leveraging microfluidic array plate (MAP) technology, the QuantStudio Absolute Q system facilitated consistent reagent distribution, filling an average of 20,465 microchambers out of the total of 20,480 microchambers available to be filled, across all 32 reactions (Figure 6). The microchamber fill rate is >99.9% (±0.1%) digitization into more than 20,000 microchambers for each reaction. When 99% of available microchambers are filled with Absolute Q 1-Step RT-dPCR Master Mix, over 95% of the input reaction mix is effectively analyzed, leaving less than 5% of the reagents wasted.

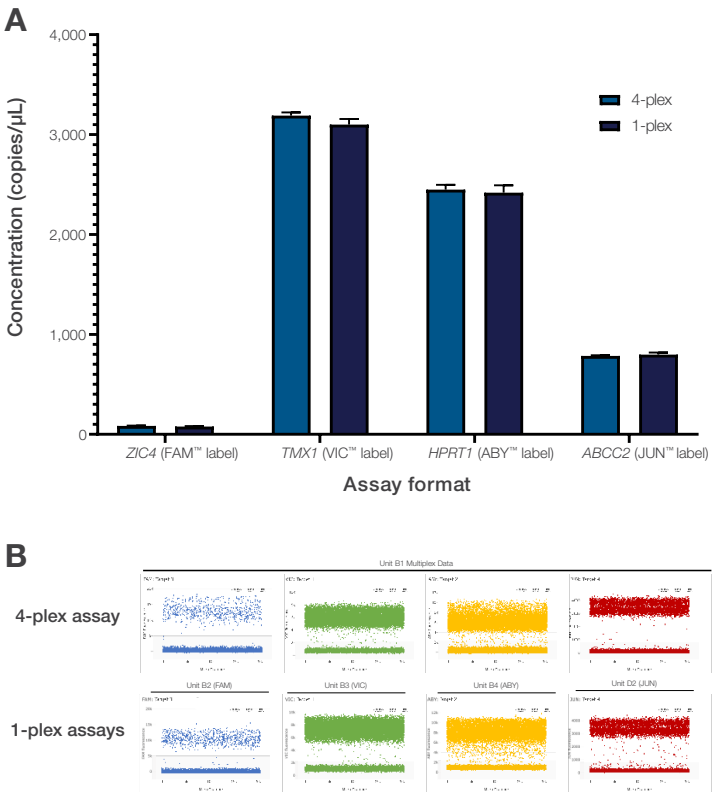

Figure 5. Reproducible quantification of RNA targets in both single-plex and multiplex dPCR assays. (A) Reported concentrations for 4 RNA targets measured using a 4-plex dPCR assay and 1-plex assays. **(B)** 1D dPCR data plots for 4-plex and 1-plex assays.

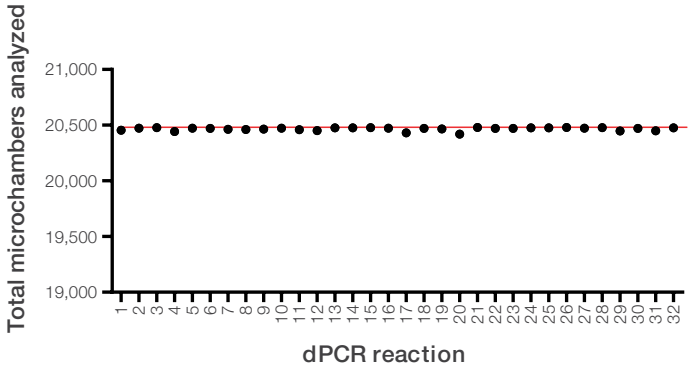

Figure 6. Microchambers accepted for analysis for 32 dPCR reactions detecting RNA targets using the Absolute Q 1-step RT-dPCR Master Mix. The red line at 20,480 reflects the total number of microchambers available per array of the QuantStudio MAP16 dPCR plate.

Stability of Absolute Q 1-Step RT-dPCR Master Mix

With a turnaround time of under 2 hours, dPCR plates may be prepared ahead of time, stored at the recommended 4°C, and saved for subsequent runs. To demonstrate the stability of the Absolute Q 1-Step RT-dPCR Master Mix, complete dPCR reactions containing the master mix, assay, and RNA template were prepared, loaded onto the QuantStudio MAP16 dPCR plate, stored at 2–8°C for up to 16 hours, and run on the QuantStudio Absolute Q Digital PCR System at 2-hour intervals. Percent difference from the baseline 0-hour measurement was calculated for each time point. To calculate percent difference, the following formula was used: Percent difference = (experimental value – baseline value)/baseline value x 100%. Quantification of the target remained consistent within 20% of the 0-hour measurement across this 16-hour time frame, demonstrating the stability of the Absolute Q 1-Step RT-dPCR Master Mix (Figure 7).

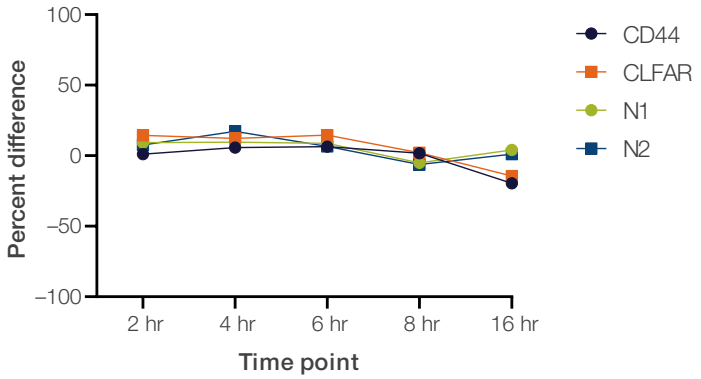

Figure 7. Effect of time on the stability of the Absolute Q 1-Step RT-dPCR Master Mix and quantitative results for 4 RNA targets. dPCR mixtures were prepared together and loaded onto QuantStudio MAP16 dPCR plates at the same time (0 hr). Plates were then stored at 2–8°C and run every 2 hours. The percent difference in quantification from the 0-hour measurement was calculated for each time point.

Inhibitor tolerance

The Absolute Q 1-Step RT-dPCR Master Mix is capable of providing robust quantification in the presence of many common PCR inhibitors. The concentrations of three targets were measured in the presence of 4 common inhibitors (hematin at 15 µM, humic acid at 10 ng/µL, tannic acid at 20 ng/µL, and ferric chloride at 50 µM) at levels that far exceed typical concentrations in purified RNA samples. To assess quantification performance in the presence of these inhibitors, the percent differences in the targets' measured concentrations, with respect to a control reaction without inhibitors, were calculated. Figure 8 shows the differences from the baseline value with no inhibitor. Concentrations remained within 30% of the results obtained with the no-inhibitor control.

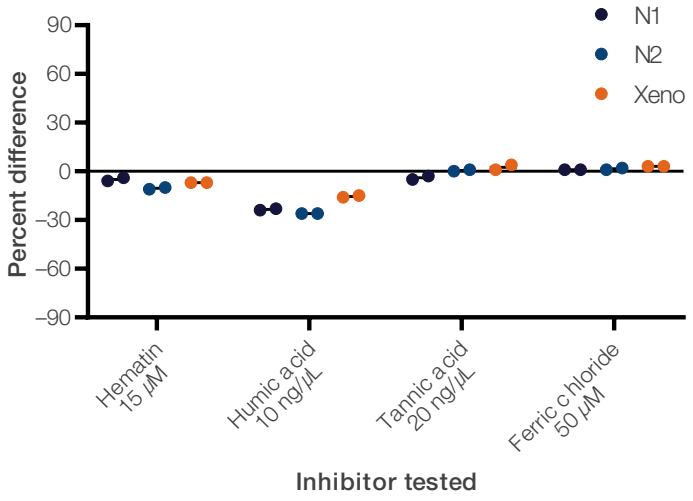

Figure 8. Percent differences in measured concentrations of three RNA targets in the presence of common PCR inhibitors.

Ordering information

Description	Quantity	Cat. No.
Absolute Q 1-Step RT-dPCR Master Mix (4X)	200 reactions	A55146
Absolute Q MAP16 Plate Kit and 1-Step RT-dPCR Master Mix	12 plates, 200 reactions	A55165

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