

Transferring dPCR assays to the QIAcuityDx[®] Digital PCR System from QIAcuity[®] One, Four or Eight instruments

Introduction

The QIAcuityDx Digital PCR System is for clinical users and diagnostic developers who seek to utilize digital PCR (dPCR) to generate clinical data in an environment where regulatory and cybersecurity compliance are becoming increasingly important. The system builds on the success of the existing QIAcuity instrumentation portfolio by streamlining clinical assay development and testing, while still delivering highly precise, absolute quantification of target DNA and RNA. This white paper guides users interested in transferring PCR assays over to the QIAcuityDx System. It also demonstrates the similarity in performance between the QIAcuityDx System and other instruments in the QIAcuity family.

Since the assays used in QIAcuity One, Four or Eight instruments are not intended for clinical use –because these instruments are not regulated as medical devices – their transfer to QIAcuityDx does not make them suitable for clinical applications. Therefore, they remain for research use only applications, from a QIAGEN perspective.

Key features of the QIAcuityDx System

Integrated QIAcuityDx software with Utility

(open) Mode: Supports custom assay development with flexible run parameters and reporting options.

QIAcuityDx Universal MasterMix Kit: A newly optimized formulation, manufactured under strict regulatory standards, with a separate vial of MgCl₂ for assay optimization.

QIAcuityDx Nanoplate 26k 24-well: A new nanoplate designed, manufactured and tested to conform to standards for use of medical-grade materials; supports

dPCR applications requiring the highest level of sensitivity, while maintaining the precision achieved from fixed nanowells in plate format.

The QIAcuityDx instrument and driving software, QIAcuityDx Universal MasterMix and QIAcuityDx Nanoplate 26k 24-well are IVD medical devices.

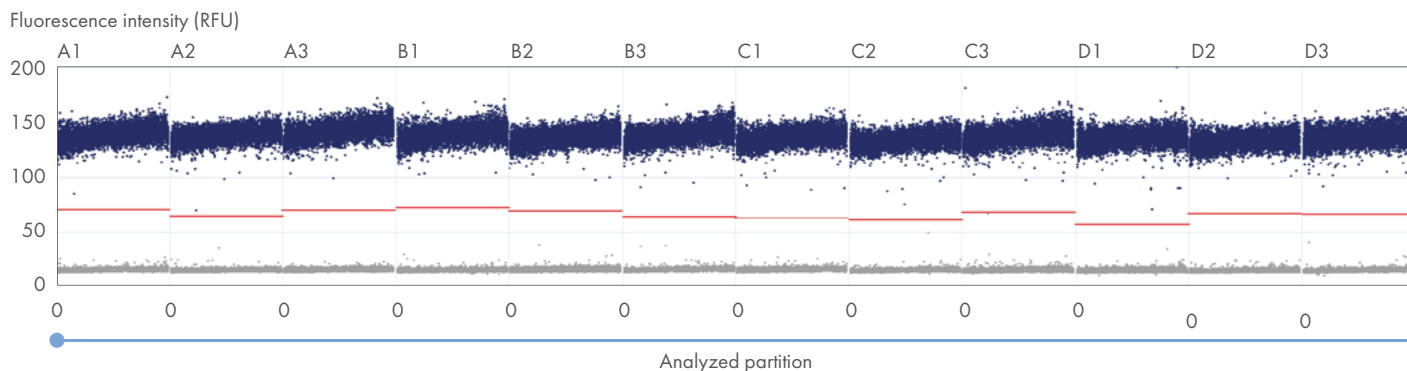
QIAcuity instrumentation

The non-medical device QIAcuity digital PCR family of instruments is functionally comparable, consisting of equivalent operational modules within the device: Handling Module, Prime-Rolling Module, Cycling Module and Imaging Module.

The key difference between the QIAcuity One, Four and Eight instruments and the QIAcuityDx Four instrument lies in the latter's calibration, design controls and manufacturing processes, which are implemented in accordance with applicable medical device regulations and ISO 13485 Medical devices – Quality management systems – Requirements for regulatory purposes. These measures support the compliance of the QIAcuityDx System as an in-vitro medical device.

When transferring an assay onto the QIAcuityDx system, an overall decrease in the obtained Relative Fluorescent Unit (RFU) values may be observed. This is due to adjustments made to the calibration process for the QIAcuityDx Imaging Module, which result in an overall reduction in RFU. However, this decrease applies to both the positive (signal) and negative (noise) partitions. ►

LS ERBB 2 Green



DX ERBB 2 Green

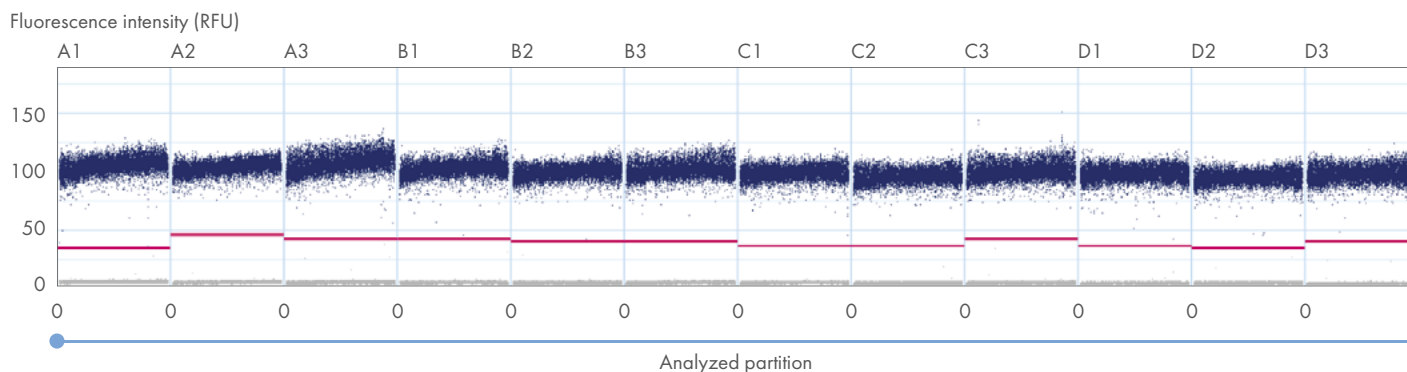


Figure 1. Absolute quantification analysis on QIAcuity and QIAcuityDx systems is highly comparable. Scatterplots for the green channel using the same reaction mix run on QIAcuity Four (top) and QIAcuityDx Four (bottom).

This means that the overall signal-to-noise ratio remains highly comparable across all **QIAGEN dPCR systems**, with no significant impact on the absolute quantification values obtained from the resulting software analysis (Figure 1).

If an increased signal is required, both exposure time and gain settings can be adjusted via the software user interface on both the QIAcuity and QIAcuityDx systems (further guidance on this can be found in the user manuals for both systems (1, 2)).

QIAcuity mastermixes

All QIAcuity digital PCR systems have specific and optimized mastermixes; in most cases, these are freely interchangeable, and users may consider testing several options before finalizing any given assay. Each mastermix has been optimized to maximize system performance during dPCR runs.

A relevant consideration when transferring an assay from the QIAcuity One, Four or Eight systems (e.g., with the QIAcuity Probe PCR Kit) to the QIAcuityDx System (e.g., with the QIAcuityDx Universal MasterMix Kit) is that each available kit has slight differences in the formulation of the mix, which may lead to subtle differences in the overall performance of the assay.

The QIAcuity Probe PCR Kit has been optimized for ease of use. It consists of an 'all-in-one' mastermix formulation in which users only require the addition of primers and probes. The QIAcuityDx Universal MasterMix Kit has been developed to support assay 'fine-tuning' through removal of $MgCl_2$ from the core mastermix. Within the QIAcuityDx Universal MasterMix Kit, $MgCl_2$ is provided in a separate tube to support assay optimization efforts by incorporating a titration step for this DNA polymerase cofactor.

When transferring an assay from any of the QIAcuity One, Four or Eight Systems to the QIAcuityDx System, it is recommended that users conduct a MgCl_2 titration run to determine the optimal MgCl_2 concentration for a given assay (Table 1).

Table 1. Example titration table for optimization of a dPCR assay

MgCl ₂ (200 mM) volume per reaction (40 µL)				
0.8 µL	1.0 µL	1.2 µL	1.4 µL	1.6 µL
4 mM	5 mM	6 mM	7 mM	8 mM

QIAcuity Nanoplate

A significant advantage of the QIAcuity family of instruments is that the nanoplate-based workflow can deliver results in ~2 hours. A streamlined qPCR-like workflow enhances the user experience over more traditional dPCR approaches by minimizing hands-on time, with plate setup as the only manual step. The following product lines are available for use on the QIAcuity systems (Table 2).

Laboratories may choose to use the QIAcuityDx Utility (open) Mode together with IVD components to develop their own Laboratory Developed Tests (LDTs) for use within their health institutions, in compliance with country- or territory-specific regulations, such as Article 5(5) of Regulation (EU) 2017/746 (IVDR) in the European Union or requirements for CLIA-licensed or accredited clinical laboratories in the United States, which allow them to develop, validate and perform its own testing services.

Table 2. Nanoplate configurations

Plate type	Frame color	No. of wells	Input volume/well (µL)	No. of partitions	Partition volume (nL)
QIAcuityDx Nanoplate 26k, 24-well	Red	24	40	Approx. 26,000	Approx. 0.82
QIAcuity Nanoplate 26k, 24-well	Blue	24	40	Approx. 26,000	Approx. 0.82
QIAcuity Nanoplate 8.5k, 24-well	White	24	12	Approx. 8500	Approx. 0.34
QIAcuity Nanoplate 8.5k, 96-well	Grey	96	12	Approx. 8500	Approx. 0.34
QIAcuity Nanoplate 26k, 8-well*	Light Blue	8	40	Approx. 26,000	Approx. 0.82

*Please note QIAcuity Nanoplate 26k, 8-well is not supported by QIAcuityDx System at the time of writing.

Incorporating IVD medical device consumables, such as the QIAcuityDx Nanoplate 26k 24-well and QIAcuityDx Universal MasterMix, in the QIAcuityDx instrument, can help reduce the validation efforts required to establish LDTs, in alignment with ISO 5649:2024 Medical laboratories – Concepts and specifications for the design, development, implementation and use of laboratory-developed tests.

QIAcuity software

The software supporting the QIAcuityDx platform adopts different architectures from those used on the QIAcuity One, Four and Eight Platforms. These architectural differences were implemented to address the differing needs of diagnostic customers compared to research users.

The non-medical device platform software offers a rapidly evolving user environment, facilitating the roll-out of cutting-edge features and solutions that support research organisations in pushing the limits of dPCR technology. In contrast, the QIAcuityDx Software Suite, intended to be used with IVD medical device assays only, was developed under a module architecture to support both user development of Laboratory Developed Tests (LDTs)/ In-House Devices (IHDs) through the use of the Utility (open) Mode embedded in the software, as well as a dedicated IVD (closed) Mode to support IVD development incorporating software plugin architecture. ▶

The IVD (closed) Mode is only available for dedicated kitted assays developed by QIAGEN or QIAGEN partners. Such assays are supported by formally completed regulatory documentation with scientific validity, analytical and clinical performance data. Each IVD Mode plugin can be developed to avoid interference with core software functionality.

The IVD (closed) Mode still delivers a dedicated user environment that can minimize data entry, incorporate secondary analytics and automate the analysis and reporting of clinically actionable data via a two-way LIS connection.

The core features and underlying analytics running in all software versions are essentially equivalent. All software versions support users in configuring dPCR runs, assigning samples to nanoplate wells and conducting core analytics (including Absolute Quantification, Mutation Detection, CNV analysis and Gene Expression analysis).

When transferring an assay from any of the QIAcuity One, Four or Eight platforms, users can only use the Utility (open) Mode within the QIAcuityDx Software Suite.

Assay considerations

The QIAcuity platforms are broadly comparable at the instrument level. However, when considering assay design, there are some differences in supported fluorophores between the instruments (Table 3).

Differences in supported fluorophores were determined based on performance optimization and verification efforts.

In turn, part of the system development strategies aim to optimize corrections to associated crosstalk between dye channels. While the QIAcuityDx System operates with all fluorophores supported by the other platforms in the QIAcuity family, crosstalk is not optimized for these fluorophores, and performance may show variability.

It is recommended that customers who anticipate transitioning from QIAcuity One, Four or Eight instruments to the QIAcuityDx Four instrument select fluorophores that are supported by both systems.

Performance equivalence

The QIAcuity platforms are broadly equivalent in overall performance (Figure 2). Extensive, documented internal studies have demonstrated that minor variations can be attributed to instrument type, mastermix type and nanoplate configuration used.

Variation between the QIAcuity and QIAcuityDx system is typically <10% when considering the systems as a whole, with the most significant proportion of this variation attributable to the nanoplate configuration used. At low copy numbers, such differences are considered to be of low significance. For example, finding 5.5 target copies versus 5 target copies is not likely to be clinically relevant.

As most of the observed variation was attributable to the choice of nanoplate configuration, we recommend selecting the appropriate nanoplate type for your chosen application as early as possible during the development process (Table 4).

Table 3. Supported fluorophores on QIAcuity and QIAcuityDx systems

Channel	Excitation wavelength (nm)	Emission wavelength (nm)	Supported fluorophores on QIAcuityDx Four	Supported fluorophores on QIAcuity One, Four and Eight
Green	463-503	518-543	FAM™	FAM™, EvaGreen®, Atto 488, Alexa Fluor® 488
Yellow	514-535	550-564	HEX™	HEX™, VIC®
Orange	543-565	580-606	TAMRA™	TAMRA™, Atto 550
Red	570-596	611-653	ROX™	ROX™, Texas Red®
Crimson	590-640	654-692	Cy5®	Cy5®, Quasar 680

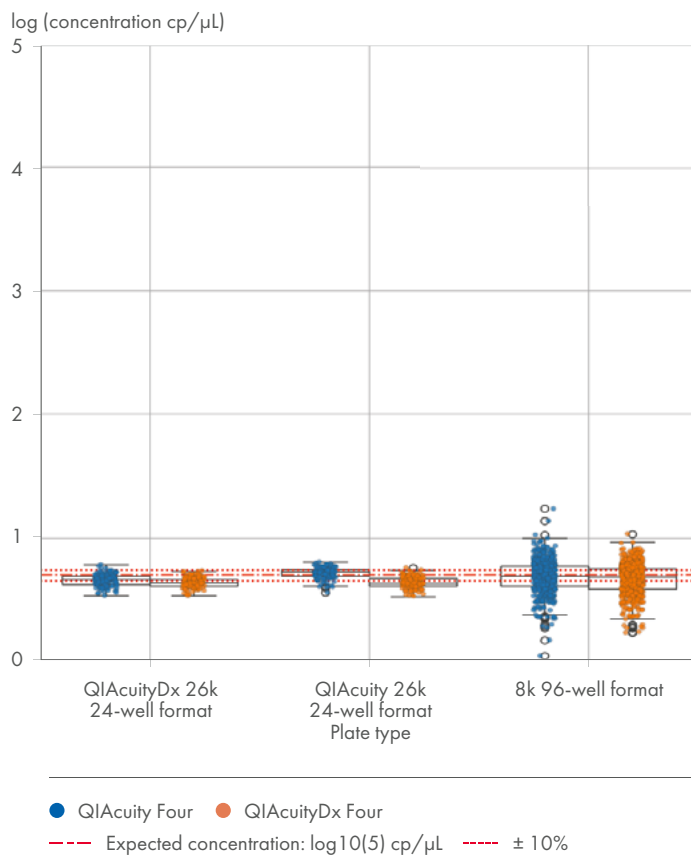


Figure 2. Equivalent performance of QIAcuity and QIAcuityDx systems.

System variability comparing a combination of nanoplate configuration and mastermix on QIAcuity Four and QIAcuityDx Four instruments. A cross-system assessment was performed using dilutions of commercially available NIST 2372a, alongside a development assay for ERBB2 labeled with a FAM probe. A low-copy template, 5 cp/μL, is displayed as representative of typical low-copy use cases for dPCR (source: development data).

Table 4. Recommended nanoplate types for common applications

Application/Use case	Nanoplate
LDT/IHD applications and IVD development	QIAcuityDx Nanoplate 26k, 24-well
Research applications/Feasibility	QIAcuity Nanoplate 26k, 24-well
High-throughput research applications	QIAcuity Nanoplate 8.5k, 96-well
Low-throughput, research applications	QIAcuity Nanoplate 8.5k, 24 well

References

1. QIAcuityDx System User Manual. HB-3567-001. July 2024.
2. QIAcuity User Manual. Version 11. August 2025.
3. QIAcuityDx User Manual. HB-3567-002. February 2025.

Ordering Information

Product	Contents	Cat. no.
QIAcuityDx Digital PCR System	A single touchpoint, in vitro diagnostic digital PCR (dPCR) system that seamlessly integrates partitioning/processing, PCR and analysis.	911060
QIAcuity One, Four or Eight Platform System	Digital PCR instrument for detecting up to 5 fluorescent dyes or up to 8 fluorescent dyes operating under software version 3.1 or higher, enabling up to 12plex analysis	911021; 911042; 911052
QIAcuityDx Nanoplate 26k 24-well	10 QIAcuityDx Nanoplate 26k 24-well, 11 QIAcuity Nanoplate Seals	260001
QIAcuity Nanoplate 26k 24-well	10 QIAcuity Nanoplate 26k 24-well, 11 Nanoplate Seals	250001
QIAcuity Nanoplate 8.5k 96-well	10 QIAcuity Nanoplate 8.5k 96-well, 11 Nanoplate Seals	250021
QIAcuity Nanoplate 8.5k 24-well	10 QIAcuity Nanoplate 8.5k 24-well, 11 Nanoplate Seals	250011
QIAcuityDx Universal MasterMix Kit	For 400 x 25 µL reactions: 4 x QIAcuityDx Universal MasterMix (5 mL), RNase-Free Water (6 x 1.9 mL)	260101
QIAcuity Probe PCR Kit	For 400 x 25 µL reactions: 4 x QIAcuity Probe Master Mix (5 mL), RNase-Free Water (6 x 1.9 mL)	250103



For more information on the QIAcuityDx Digital PCR System, visit: www.qiagen.com/qiacuitydx



For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use or the user operator manual. QIAGEN instructions for use and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services (or your local distributor). All figures and underlying data are from the QIAGEN R&D Department.

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