

Real-time PCR

The QuantStudio IVD real-time PCR ecosystem

State-of-the-art solutions for faster molecular diagnostic (MDx) assay design and development

Introduction

Tailored molecular assays have revolutionized the MDx sector and reduced turnaround times for the detection of cancer, infectious disease agents, and inherited conditions with laboratory, clinical, and patient samples. Quantitative PCR (qPCR), also known as real-time PCR, is widely used in the MDx field to detect disease-related biomarkers because it enables accurate, fast, and highly specific quantitative analysis of molecular targets.

The general workflow for an MDx assay includes three stages: nucleic acid sample preparation, PCR amplification and quantitative detection of specific targets, and data analysis. Protocols and tools for nucleic acid sample preparation vary depending on the biological starting material. Once nucleic acid is extracted from a sample, a variety of MDx assays can be used to amplify and detect the target(s) of interest.

The QuantStudio Dx real-time PCR ecosystem

The Applied Biosystems™ ecosystem of QuantStudio™ Dx real-time PCR instruments, reagents, consumables, and software is designed to address specific challenges and obstacles experienced by MDx assay developers. We offer solutions for every MDx qPCR application, including qualitative pathogen detection, quantitation of viral load, single nucleotide polymorphism (SNP) analysis, and detection of antibiotic and antiviral drug resistance. The QuantStudio Dx real-time PCR ecosystem also has integrated features for effortless regulatory compliance, to streamline approval of novel therapeutics and reduce time-to-market.

Overcome hurdles in MDx assay development with the QuantStudio Dx real-time PCR ecosystem

Robust MDx assays must meet high quality and performance specifications for accuracy, reproducibility, specificity, and sensitivity. An MDx assay must enable highly specific target detection; detection of low copy number targets; reliable performance in the presence of PCR inhibitors; benchtop stability throughout processing; and multiplexing capabilities. Manufacturing consistency and regulatory compliance are also essential.

Technical challenges are commonly encountered in the amplification and detection stages of the MDx workflow, and they must be dealt with to optimize assay performance. Applied Biosystems™ qPCR plastics and consumables, TaqPath qPCR master mixes, and QuantStudio Dx instruments have been designed with these challenges in mind. The QuantStudio Dx real-time PCR ecosystem can help MDx developers overcome some of the hurdles in assay development and enjoy excellent quality, reproducibility, and consistency.

Specific target detection

MDx assays must allow accurate, specific, and sensitive detection of one or more genetic targets. RNA and DNA targets can be detected with high specificity and sensitivity using Applied Biosystems™ TaqPath™ 1-Step RT-qPCR Master Mix, CG (Figure 1A). TaqPath™ qPCR Master Mix, CG, enables specific and sensitive detection of cDNA targets in MDx assays (Figure 1B). TaqPath™ ProAmp™ Master Mix allows more accurate genotyping than other commercially available master mixes. It enables highly specific allelic discrimination, as indicated by excellent cluster separation and high resolution in genotyping plots (Figure 1C).

TaqPath master mixes support MDx target detection over a dynamic range that spans at least 6 orders of magnitude. A wide dynamic range is particularly important for microbiology laboratories that analyze viral and bacterial RNA and DNA targets. TaqPath™ BactoPure™ Microbial Detection Master Mix helps minimize nonspecific amplification of background DNA to maximize specificity. A maximum C_q of 40 was observed only with the TaqPath BactoPure master mix in tests conducted with it and three other commercially available master mixes (Figure 1D).

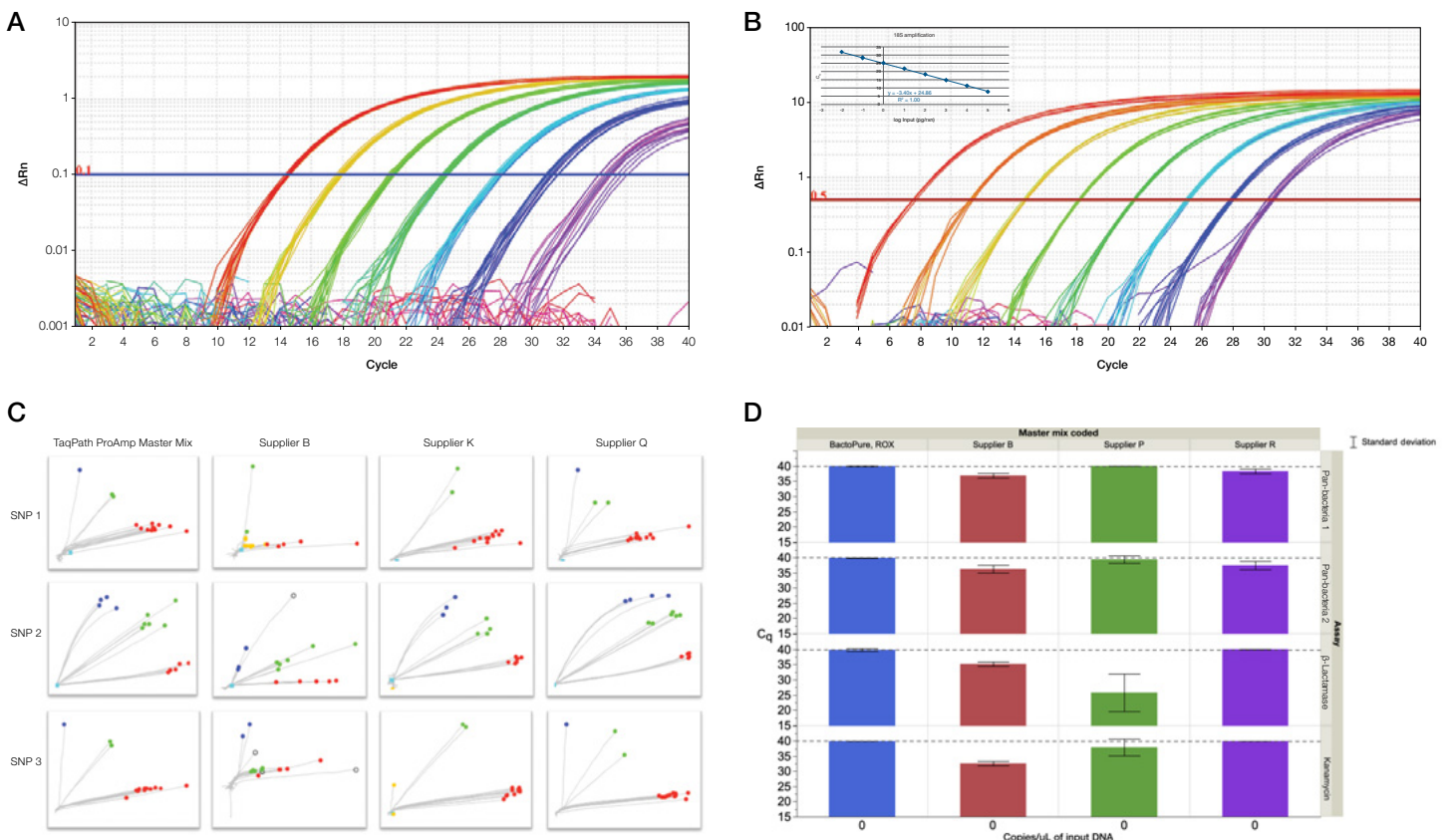


Figure 1. TaqPath master mixes enable high specificity for MDx targets, along with wide dynamic ranges, low background, and excellent allelic discrimination. (A) Amplification plots for a dilution series of poliovirus RNA amplified using TaqPath 1-Step RT-qPCR Master Mix, CG, showing a wide dynamic range for RNA detection. (B) Representative amplification plots obtained with TaqPath qPCR Master Mix, CG, showing specific detection of an 18S rRNA target in a human cDNA dilution series. (C) Three SNPs were assessed in genotyping assays using crude lysates prepared from blood samples and then diluted 20-fold. In a comparison of TaqPath ProAmp Master Mix and three other commercially available master mixes, the clearest allelic discrimination was observed with TaqPath ProAmp Master Mix. (D) Background levels of nucleic acid were lower with TaqPath BactoPure Microbial Detection Master Mix than those detected using three other commercially available master mixes.

Low copy number target detection

Targets in many MDx assays have low copy numbers or titers. TaqPath 1-Step RT-qPCR Master Mix and TaqPath qPCR Master Mix enable reliable detection of RNA and DNA targets, respectively, in samples containing as few as 10 target copies (Figures 2A, 2B). Pan-bacterial, pan-viral, pan-mammalian, and mastadenovirus targets can be detected at concentrations as

low as 1 copy/ μL using TaqPath BactoPure Microbial Detection Master Mix, without nonspecific amplification of the control (Figure 2C). TaqPath BactoPure Microbial Detection Master Mix can be used as a diagnostic tool for detecting acute or low-level microbial infections or as a quality control (QC) tool for detecting microbial contaminants in nonmicrobial MDx assays.

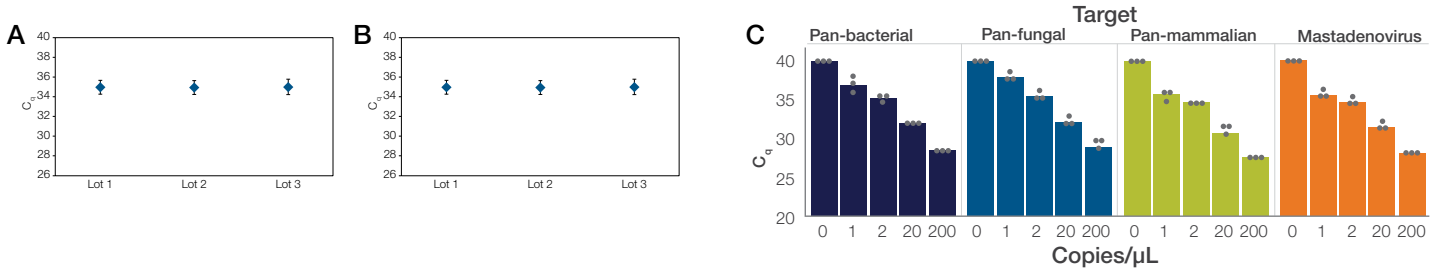


Figure 2. Detection of targets with low copy numbers using TaqPath master mixes. (A) Samples containing 10 copies of an RNA target were amplified in an assay with an *RNase P* control using three different lots of TaqPath 1-Step RT-qPCR Master Mix, CG. (B) Samples containing 10 copies of a human DNA target were amplified using three different lots of TaqPath qPCR Master Mix, CG, in an assay with an *RNase P* control. (C) Known concentrations of synthetic DNA containing the targets of interest were diluted in TE buffer and accurately detected using TaqPath BactoPure Microbial Detection Master Mix.

PCR inhibitor tolerance

PCR inhibitors are often found in reaction mixtures after sample preparation and other steps in the MDx workflow. The unique TaqPath master mix formulations deliver robust performance in the presence of common PCR inhibitors like heparin, hematin, humic acid, and IgG. Targets were amplified in the presence of PCR inhibitors, using TaqPath master mixes and

other commercially available master mixes, and the ΔC_q values were evaluated. The PCR inhibitors did not significantly impact amplification or detection in reactions prepared with TaqPath master mixes (Figure 3). Our results confirm that TaqPath master mixes are more tolerant of PCR inhibitors than other commercially available products.

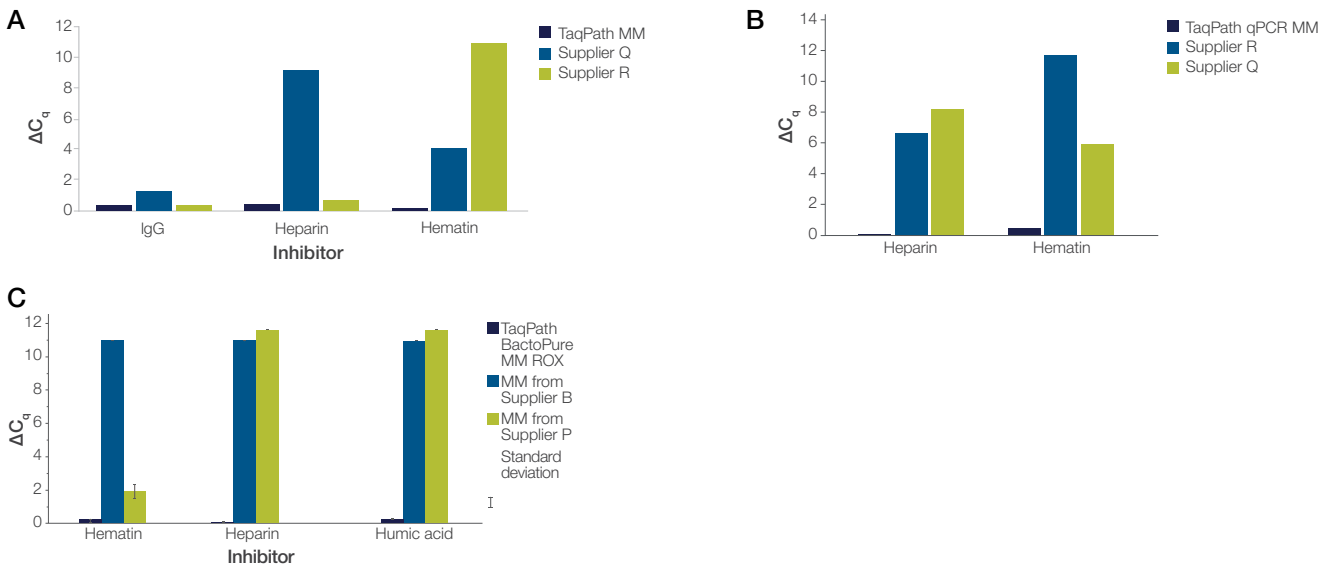


Figure 3. TaqPath master mixes are formulated to help ensure performance in the presence of PCR inhibitors. (A) ΔC_q observed in the presence of IgG (1.2 $\mu\text{g}/\text{reaction}$), heparin (0.04 U/reaction), or hematin (30 μM). The smallest ΔC_q values were observed in reactions prepared with TaqPath master mix. (B) ΔC_q of qPCR reactions prepared with TaqPath qPCR Master Mix, CG, and master mixes from two other vendors, in the presence of heparin (5×10^{-4} U/ μL) and hematin (30 μM). The TaqPath master mix was far more tolerant of both inhibitors. (C) ΔC_q of a pan-bacterial qPCR assay prepared using TaqPath BactoPure Microbial Detection Master Mix and master mixes from two other vendors, in the presence of hematin (30 μM), heparin (1×10^{-3} U/ μL), or humic acid (30 ng/ μL). The TaqPath master mix enabled better performance in the presence of each inhibitor.

Benchtop stability

MDx assays often have multistep workflows that may require non-negligible hands-on time. TaqPath qPCR Master Mix, CG, is formulated with a thermostable polymerase that promotes

benchtop stability at room temperature to help ensure consistency throughout processing (Figure 4).

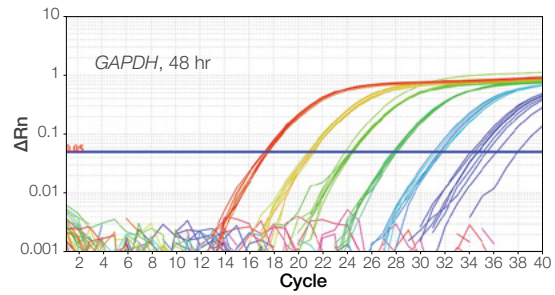
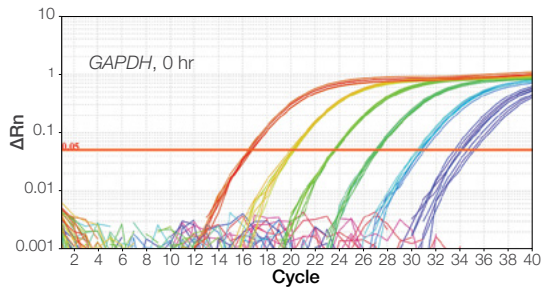


Figure 4. TaqPath qPCR Master Mix, CG, remains stable over time at ambient temperature. qPCR of a *GAPDH* target gave similar results at time 0 and after 48 hours at 24°C.

Multiplexing capability

MDx researchers often analyze multiple targets simultaneously to maximize efficiency and throughput. With Applied Biosystems™ MicroAmp™ 96- and 384-well plastic consumables and loading plates for QuantStudio Dx systems, TaqPath master mixes can be

used to analyze a variety of sample types for multiple targets—including exogenous and endogenous controls—in one assay (Figure 5).

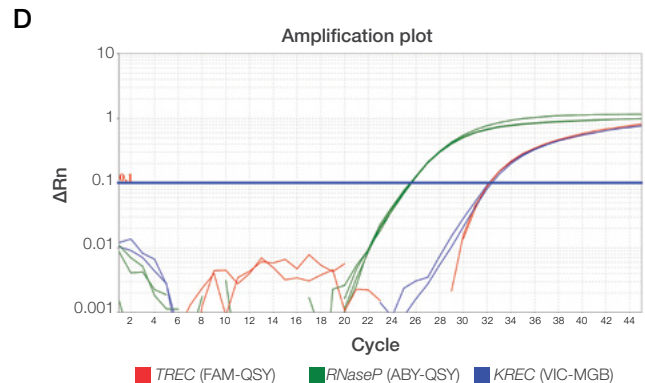
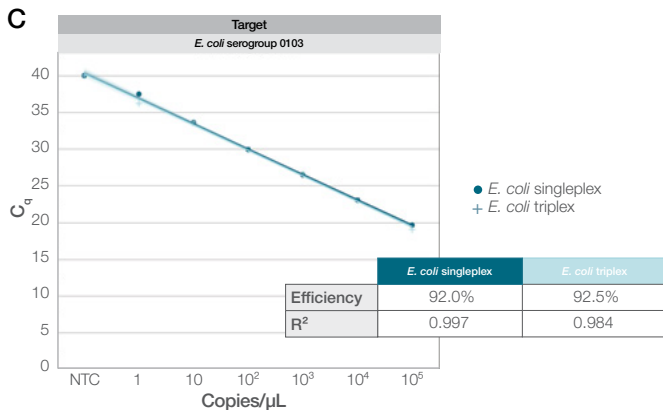
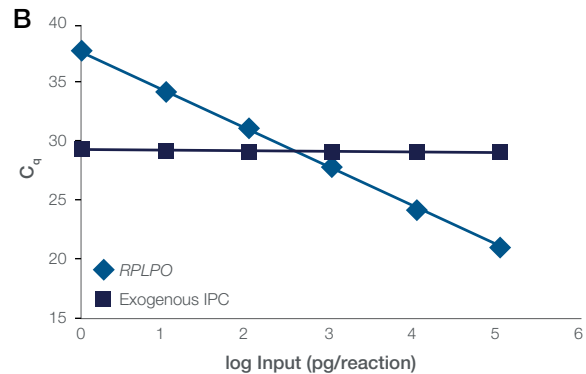
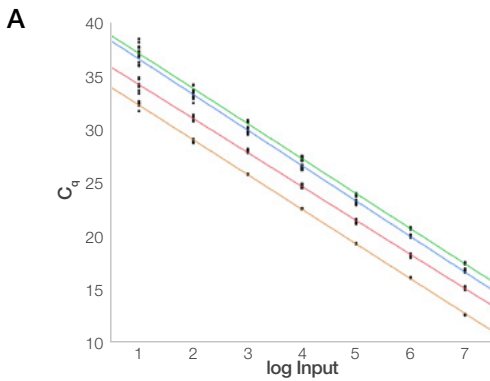
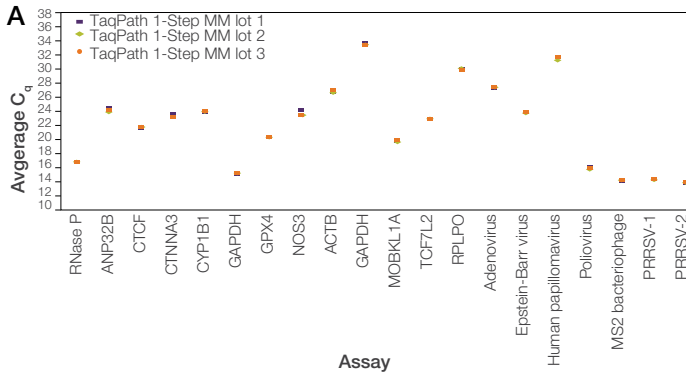


Figure 5. TaqPath master mixes are optimized for multiplexing. **(A)** Amplification of human cDNA in a four-plex reaction prepared with Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix. Each color represents a different gene target. **(B)** Amplification of human cDNA targets in a duplex reaction prepared with TaqPath qPCR Master Mix, CG, to detect an *RPLPO* gene target and an exogenous internal positive control (IPC). **(C)** Amplification of a *wzy* gene target from enterotoxigenic *E. coli* in singleplex and triplex reactions prepared using TaqPath BactoPure Microbial Detection Master Mix with ROX dye. **(D)** Amplification curves for *TREC*, *RNase P*, and *KREC* DNA targets detected with individually labeled probes. Reactions were prepared using TaqPath ProAmp Master Mix.

Manufacturing consistency

MDx assays require reagents that work consistently from lot to lot. TaqPath master mixes are manufactured in an ISO 13485–certified facility to help ensure high quality and robust lot-to-lot



consistency. The same target amplification levels are observed when qPCR is performed with different lots of a given TaqPath master mix regardless of sample input (Figure 6).

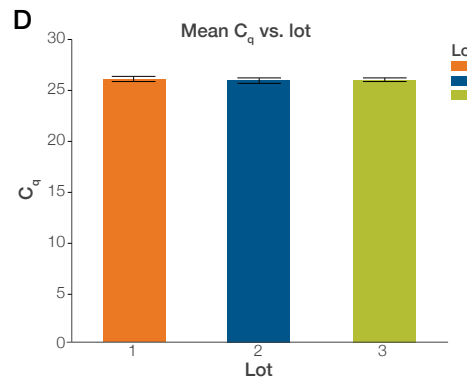
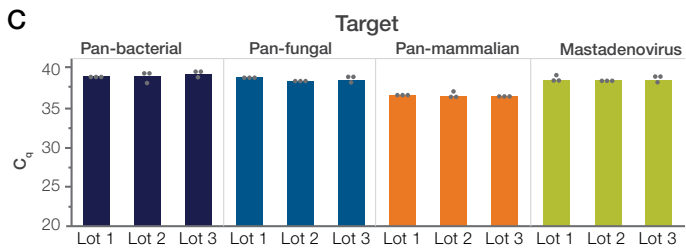
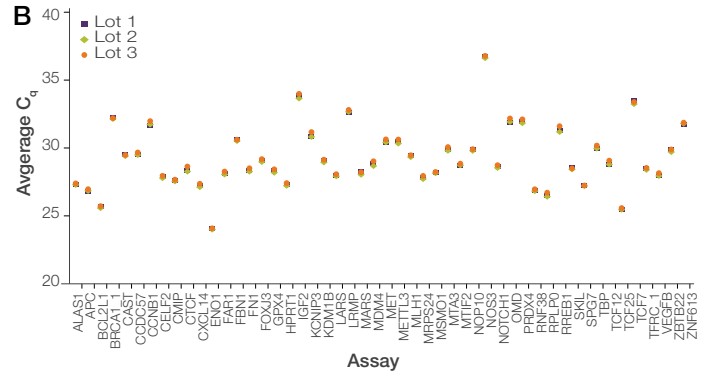


Figure 6. TaqPath master mixes deliver lot-to-lot consistency. (A) C_q values for amplification of targets in total human and viral RNA using three different lots of TaqPath 1-Step RT-qPCR Master Mix, CG. (B) C_q values for amplification of targets in human cDNA using three different lots of TaqPath qPCR Master Mix, CG. (C) Amplification of DNA templates for specific pan-bacterial, pan-fungal, pan-mammalian, and mastadenovirus targets with three different lots of TaqPath BactoPure Microbial Detection Master Mix. (D) Amplification of genomic DNA with three different lots of TaqPath ProAmplification Master Mix.

Flexible and streamlined assay development with TaqMan predesigned assays and custom primers and probes

Our portfolio of custom fluorophores and quenchers provides unprecedented flexibility for assay development with the quality, performance, and reliability of gold-standard Applied Biosystems™ TaqMan™ assay chemistry. Our probes deliver outstanding signal-to-noise ratios and reproducibility. They are synthesized using the same raw materials and manufacturing know-how as predesigned TaqMan Assays, which have been cited in over 300 patents and more than 200,000 publications. In addition, our ISO 13485–certified manufacturing facility and processes offer the traceability you need to satisfy regulatory requirements.

Streamline data analysis with Diomni Software

Software that helps make your workflow more efficient from sample to results

Applied Biosystems™ Diomni™ Software can be seamlessly integrated into qPCR workflows for efficient and automated data interpretation and reporting. Automated QC and data interpretation with Diomni Software reduces data review time by more than 2 hours, and you can have results within about 3 hours. The plug-and-play assay design feature reduces setup time by incorporating digital instructions for use into the assay file. With Diomni Software integration, data can be automatically transferred to an information management system or shared.

Assay development support

We help our partners bring their MDx assays to market by offering full support, services, and insight throughout the MDx development workflow from the bench to commercialization. Our Original Equipment Manufacturer (OEM) and Commercial Supply teams provide personalized support, industry and technology perspectives, reagent customization, analytical validation consulting services, and more.

Global Customer Concierge Services

In addition to the QuantStudio Dx real-time PCR ecosystem, we offer on-site orientation, the AB Platinum instrument service plan, instrument hardware qualification services, and digital remote support to help ensure seamless implementation of our systems in your laboratory.

SmartStart Orientation—SmartStart Orientation can help your team become proficient with your QuantStudio Dx real-time PCR system and software quickly. The interactive one-day orientation is led by an experienced field application specialist who will introduce you to the instrument hardware, software, and consumables.

AB Platinum instrument service plan—With the AB Platinum plan, you can maintain optimal instrument performance and maximize uptime with personalized technical support. If your instrument needs repair, a field service engineer can be at your location within one business day.*

Instrument hardware qualification services—We offer installation qualification, operational qualification, and instrument performance qualification and verification services to verify and document that your instrument is meeting manufacturer design specifications for performance. We also offer qualification services after planned maintenance visits and any major repairs.

Digital remote support—Our network of over 2,000 trained professionals can quickly and proactively troubleshoot, diagnose problems, and help maintain the performance of your instruments. We can help you identify and possibly resolve issues remotely in minutes, so you can avoid on-site repairs that could keep you idle for days.

* Rapid response on-site support by the next business day is subject to regional availability.

Reliability and the flexibility to pivot your program

The QuantStudio Dx real-time PCR ecosystem includes all the tools, support, and services you need for MDx assay development. TaqPath master mixes enable sensitive and highly specific target detection, even targets with low copy numbers, with robust inhibitor tolerance, good benchtop stability, multiplexing capability, and manufacturing consistency. QuantStudio Dx real-time PCR systems can be seamlessly integrated with Diomni Software for streamlined data analysis and sharing. When you partner with us, you'll have access to industry-leading technical service and customer support. With the QuantStudio Dx real-time PCR ecosystem, you can also expand your research and assay development capabilities.

Infectious microbe testing

- Microbial nucleic acids
- Sexually transmitted infections
- Gastrointestinal microbes
- Infectious respiratory pathogens, including SARS-CoV-2
- Other microbial infections (e.g., tuberculosis, hepatitis, nosocomial infections)

Oncology

- Genetic testing
- Cancer screening
- Diagnosis and staging

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TaqPath master mixes are for laboratory use. It is the customer's responsibility to ensure that the performance of the product is suitable for customer's specific uses or applications. The QuantStudio 5 Dx Real-Time PCR System, QuantStudio 7 Pro Dx Real-Time PCR System, and Diomni Software are for *in vitro* diagnostic use. © 2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a trademark of Roche Molecular Systems, Inc., used under permission and license. **COL35530 0323**