April 2022

Microbial DNA dPCR Handbook

dPCR Microbial DNA Detection Assays

For digital PCR-based profiling/detection of microbial species, viruses, antibiotic resistance genes, or virulence factor genes

Sample to Insight

QIAGEN

Contents

Kit Contents

dPCR Microbial DNA Detection Assay	
Catalog no.	**250207: DMA#####-(F/H/T/R/C)**
No. of reactions	**200***
Microbial DNA Detection Assay (tube)	1
Lyophilized Assay (tube)	1

* 200 rxn in 40 µl (Nanoplate 26k); 666 rxn in 12 µl (Nanoplate 8.5k)

dPCR instruments compatible with the assay portfolio

Type	**Instruments**	**Nanoplate formats**	**Instrument cat. nos.**
2 channels	QIAcuity 1-2plex	96-well (96LV) 24-well (24HV)	911001
5 channels	QIAcuity 1-5plex	96-well (96LV)	911021
	QIAcuity 4-5plex	24-well (24HV)	911042
	QIAcuity 8-5plex		911052

dPCR Mastermixes compatible with the assay portfolio

Name	**Description**	**Mastermix cat. nos.**
QIAcuity Probe PCR Kit	Standard Mastermix for probe-based assays in nanoplates on the QIAcuity	250101, 250102, 250103
QIAcuity One-Step Advanced Probe PCR Kit	1-step RT-PCR Mastermix optimized for the quantification of RNA and DNA targets in nanoplates on the QIAcuity	250131, 250132

Shipping and Storage

dPCR Microbial DNA Detection Assays consist of 2 primers and one hydrolysis probe that are lyophilized in a single tube. The lyophilized primer-probe mix is shipped at RT (room temperature) and should upon receipt be stored protected from light at –30 to –15°C in a constant-temperature freezer. Under these conditions the assays are stable, without showing any reduction in performance and quality, until the date indicated on the label. Before usage, the lyophilized primer-probe mix has to be dissolved. Before opening the tube, centrifuge the tube briefly to collect all material at the bottom of the tube. To dissolve the primer-probe mix, add 400 µl of sterile, nuclease-free TE buffer; mix; and leave for 20 minutes to allow the primer-probe mix to completely dissolve. We do not recommend dissolving primers and probes in water. They are less stable in water than in TE buffer, and some may not dissolve easily in water. For short term storage, the dissolved dPCR Assay mix can be stored at 2–8°C. Repeated freeze-thaw cycles should be avoided by storing in aliquots.

Intended Use

The dPCR Microbial DNA Detection Assays are intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of dPCR Microbial DNA Detection Assay is tested against predetermined specifications to ensure consistent product quality.

Introduction

Bacteria, fungi, viruses, and parasitic metazoa are ubiquitous in the environment and have adapted to various ecological niches, including the human body. This microbial diversity includes organisms that together bring a wide variety of harmful and beneficial properties to humans. They are part of all aspects of human life, from human health to food production. Research and developments in recent years have described the diverse physiological capabilities of microbes and the complexity of their lives as they interact with their environment. For example, the human microbiome found on and in our bodies has been estimated to include 10 times more microbes than human cells in the human body. Each microbe, in turn, can trigger or influence specific physiological processes. This makes the specific detection and monitoring of microbes an important tool for understanding their biological function, particularly in the context of infection or colonization of the human body.

DNA-based methods have proven to be effective for the detection and identification of both microbial species and microbial genes. dPCR Microbial DNA Detection Assays are screening tools for rapid profiling and identification of microbial species (bacteria, fungi, viruses, and parasitic metazoa), antibiotic resistance genes and virulence genes. The dPCR Microbial DNA Detection Assay portfolio covers a wide range of target species from different application fields including microbes linked to wastewater, infectious diseases, human pathogens, the human microbiome, multiple drug resistance, sepsis, food production, and environmental samples.

dPCR Microbial DNA Detection Assays provide a method for obtaining specific, accurate, and reproducible results for an individual or related group of microbial species, antibiotic resistance genes, or virulence factor genes. Every assay has been bench-verified and is ready-to-use for bacterial species or gene detection. The possibility to select each assay with any of the five fluorescent dyes FAM, HEX, TAMRA, ROX, or Cy5 enables to combine up to 5 targets in a single multiplex dPCR reaction on the QIAcuity.

dPCR Microbial DNA Detection Assays for identification of microbial taxa

Microbial DNA Detection Assays detect different microbial species that may be found in a particular sample. By combination of individual assays with up to 5 differently labelled probes (FAM, HEX, TAMRA, ROX, and Cy5), the assays can be used for profiling the microbial composition of that sample. Since the presence or abundance of different microbial species can exert profound influences on health and disease, absolute quantification of these species is a prerequisite in order to determine microbe-disease associations. Using at least one control and one experimental sample, the dPCR Microbial DNA Detection Assays can (I) determine the microbial composition at a particular body site, (II) how this composition changes over time, (III) how it compares to other populations, and (IV) how it changes due to treatments. These capabilities enhance and enable the discovery of novel microbial biomarkers.

To perform a microbial identification experiment, at least one experimental sample and one No Template Control (Microbial DNA-Free Water) sample are required. The dPCR Microbial DNA Detection Assays can be used with a variety of sample types such as isolated bacterial colonies, blood culture, swabs, stool, and other metagenomic samples.

dPCR Microbial DNA Detection Assays for identification of microbial genes

Microbial DNA detection assays detect various microbial virulence and resistance genes that may be present in a particular sample. By combining individual assays with up to 5 differentially labeled probes (FAM, HEX, TAMRA, ROX, and Cy5), the assays can be used to profile the composition of antibiotic resistance and virulence genes in a sample. The monitoring of different resistance and virulence genes is not only important for the detection of dangerous hospital pathogens with multiple drug resistances but rather a prerequisite for a deeper understanding and better control of dangerous microorganisms. The dPCR Microbial DNA Detection Assays in combination with digital PCR enhances this goal by absolute quantification and specific detection of a large set of microbial virulence and resistance genes. Using at least one control and one experimental sample, the dPCR Microbial DNA Detection Assays can (I) determine the composition of resistance and virulence genes at a particular sampling site, (II) how this composition changes over time, (III) how it compares to set standards and (IV) how it

changes due to antimicrobial treatments or altered environmental conditions. These capabilities enhance and enable the development of novel control strategies for microbial virulence and resistance.

To perform a microbial identification experiment, at least one experimental sample and one No Template Control (Microbial DNA-Free Water, cat. no. 38132) sample are required. The dPCR Microbial DNA Detection Assays can be used with a variety of sample types such as isolated bacterial colonies, blood culture, swabs, stool, and other metagenomic samples.

dPCR Microbial DNA Detection Assays for detection of microbial taxa and microbial genes

Detection of a target microbial species or gene in the sample requires differentiation of a positive sample from a negative sample. Although the dPCR Microbial DNA Detection assays are designed for high specificity, the no template controls occasionally show single positive partitions. This background signal must be taken into account when determining a positive sample.

We recommend to consider a sample as positive for the target of the respective assay if:

The mean number of detected copies/µl$^{(Sample)}$ > Limit of Blank + 1.645x standard deviation of the detected copies/µl$^{(Sample)}$)

Note: To ensure statistical significance for positive calls, it is recommended to perform 3 NTC replicates and to use the 24-well 26K nanoplate.

Note: An example calculation for determining a positive sample based on a comparison to the NTC sample is shown in Appendix A: Data Analysis, page 31.

Principle and procedure

The dPCR Microbial DNA Detection Assay portfolio is supposed to be used in nanoplate digital PCR. Digital PCR (dPCR) uses the procedure of end-point PCR but splits the PCR reaction into many single partitions, in which the template is randomly distributed across all available partitions. After PCR, the amplification target is detected by measuring the fluorescence – of either sequence-specific DNA probes or intercalating dyes – in all positive partitions. As the template is distributed randomly, Poisson statistics can be used to calculate the average amount of target DNA per valid, analyzable partition. The total amount of target DNA in all partitions of a well is calculated by multiplying the amount of average target DNA per partition with the number of valid partitions. Calculation of target concentration is determined by referring to the volume in all analyzable partitions, that is, partitions which were filled with reaction mix. The total number of filled partitions is identified by a fluorescent dye present in the reaction mix itself. Absolute quantification by dPCR eliminates the need for standard curves to determine amounts of target DNA in a given sample.

Each assay of the portfolio is based on an endpoint PCR amplification of a species-specific genetic region of the relevant microbe, or a region of an individual microbial gene. The amplified product is detected using target-specific fluorescent hydrolysis probes, which helps to improve the specificity of the assay. Assays for detection of bacterial species target the ribosomal RNA genes, mainly the 16S ribosomal RNA gene and were designed using the GreenGenes database for 16S sequences and type strain DNA sequences deposited at NCBI. Assays for fungal, viral, and metazoan species target different target-specific genetic regions including ribosomal RNA genes and other individual marker genes, each deposited at NCBI. Various databases were used for the design of assays for antibiotic resistance genes (lahey.org, ARDB, etc.) and virulence factor genes (VFDB).

For relative profiling applications, host genomic DNA (if applicable) and overall bacterial or fungal load can be measured. This can be done in a multiplex reaction in the same well of the dPCR nanoplate or in separate 1-plex reactions using the same input sample template. The dPCR Microbial DNA Assay portfolio contains a number of PAN assays for broad specificity

towards bacteria or fungi (listed in the *dPCR Microbial DNA Detection Assays Technical Information*, **www.qiagen.com/PROM-20556**). Inclusion of these assays allows the user to normalize the number of target molecules in the sample against certain background targets using the measured copies/µl values.

For identification of microbial species or microbial genes, a positive call is based on the copies/reaction count, where three No Template Control (NTC) samples are used to normalize for assay background due to various factors.

For each single reaction in the dPCR Microbial DNA Detection workflow, start with at least 5 ng of genomic DNA isolated from a metagenomic sample or as low as 2.5 ng DNA from an isolated bacterial colony. For example, 10 ng of *Escherichia coli* gDNA corresponds to ~2,000,000 copies of a single-copy gene. This number increases for multi-copy genes and also might differ in other microbial organisms.

For the dPCR reaction, prepare a premix of your DNA and the 4x QIAcuity Probe Mastermix. One premix for all replicates can be prepared. Aliquot the premix into individual wells of a preplate containing the pre-dispensed primer-probe mix. For each sample 3 reactions are set up.

After the dPCR run, the QIAcuity Software Suite enables you to inspect the dPCR results and export the copies/µl values for 2nd level analysis.

Note: Example calculations of dPCR data files are shown in Appendix A: Data Analysis, page 31.

For detailed information on the different analysis options and applied calculations refer to the *QIAcuity User Manual* on **www.qiagen.com**.

The dPCR Microbial DNA Detection Assays provide accurate and verifiable results using a variety of sample types. dPCR Microbial DNA Detection Assay Kits have been tested using genomic DNA from stool, sputum, tooth plaque, isolated bacterial colony, vaginal swab, and sewage samples and can be used with a variety of other sample types. The procedure can be performed using 5 ng genomic DNA from metagenomic samples or 2.5 ng from an isolated bacterial colony per single reaction. Genomic DNA is added to the ready-to-use QIAcuity Probe Mastermix plus Microbial DNA-Free Water (UCP water) and aliquoted into each well of the dPCR preplate, which contains pre-dispensed sets of primers and hydrolysis probes. From the pre-plate the reaction mixes are transferred to the wells of a dPCR nanoplate which is then sealed and transferred to the QIAcuity dPCR instrument (Figure 1). The following steps, partitioning, cycling, and imaging are done fully automated by the QIAcuity dPCR instrument following the preset parameters. Depending on the cycling protocol the results of the dPCR run can be analyzed in the QIAcuity Software Suite after ~2 hours.

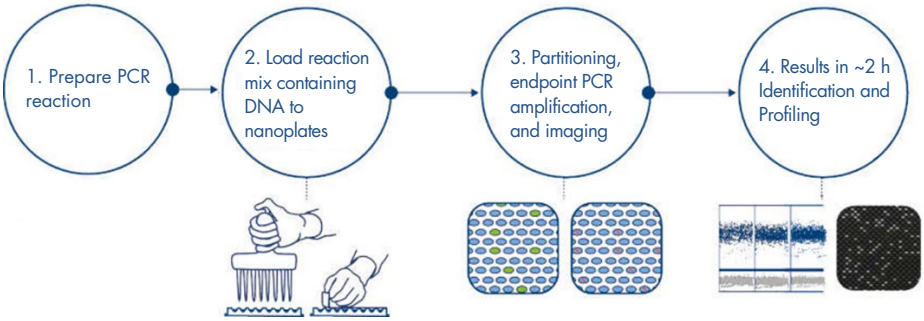

Figure 1. Overview of the dPCR Microbial DNA Detection Assay procedure. The simple workflow involves (1) the preparation of the PCR reaction by mixing the microbial genomic DNA from the sample of interest with ready-to-use QIAcuity Probe Mastermix, (2) loading the reaction mix into each well of the dPCR nanoplate and sealing, (3) performing the dPCR reaction in the QIAcuity, and (4) analyzing the data using the QIAcuity Software Suite or manually using the Excel® compatible export files of the QIAcuity Software Suite.

Assay Bundles

Each assay of the portfolio can be ordered in any of the 5 dyes FAM, HEX, TAMRA, ROX, and Cy5. This allows the combination of up to 5 individual assays in one dPCR reaction, a so-called multiplex reaction. Multiplex applications are relevant, for example, when several target organisms need to be detected and quantified in one sample and/or the available sample amount is limited. We have wet-lab tested a number of assay bundles that target marker organisms or genes of different application fields including the human microbiome, probiotics, infectious diseases, wastewater, virulence genes, resistance genes, and food production. For a list of assay bundles tested in the wet lab, see the assay product page (**www.qiagen.com/dpcr-microbial-dna-detection-assays**). To prepare a multiplex reaction, the corresponding assays are added together to the reaction mixture.

Control Assays

The dPCR Microbial DNA Detection Assay portfolio entails Pan assays that are designed to detect a broad range of bacterial or fungal species.

Two different Pan Bacteria designs are included to increase the coverage of bacterial species detected. Furthermore, Pan-Aspergillus/Candida and Pan-Aspergillus/Penicillium detect fungal species of Aspergillus and Candida, and Aspergillus and Penicillium genera, respectively. The Pan Assays can be used to monitor proper DNA extraction of bacteria and fungi. Also, they can be used to determine the overall bacterial, fungal, Aspergillus/Candida, and Aspergillus/Penicillium load in a sample.

No Template Control and Microbial DNA Positive Control can be used to ensure that the experimental conditions and PCR setup are correct. See Appendix B: Microbial DNA Positive Control (PMCv2), page 35, for more information.

QIAcuity Probe PCR Kit

The dPCR Microbial DNA Detection Assay Portfolio works in conjunction with the QIAcuity Probe PCR Kit. The QIAcuity Probe PCR Kit contains a 4x concentrated, ready to use Master Mix optimized for microfluidic use in the QIAcuity Nanoplates. This special master mix enables accurate quantification of up to 5 targets having widely differing abundance in a well of the QIAcuity Nanoplate. This saves time, money, and reduces the amount of sample material needed. Moreover, the duplex or multiplex PCR data obtained is comparable with that obtained from a singleplex PCR.

The QIAcuity Probe PCR Kit delivers singleplex or multiplex, cDNA or gDNA analysis with the highest specificity because of a novel, antibody-mediated, hot-start mechanism. At low temperatures, the QuantiNova DNA Polymerase is kept in an inactive state by the QuantiNova Antibody and a novel additive, QuantiNova Guard, that stabilizes the complex. This improves the stringency of the hot-start and prevents extension of nonspecifically annealed primers and primer dimers. Within 2 minutes of raising the temperature to 95°C, the QuantiNova Antibody and QuantiNova Guard are denatured, and the QuantiNova DNA Polymerase is activated, enabling the PCR amplification.

The QIAcuity Probe PCR Mastermix can be stored at 30°C for up to 100 hours without impairing the performance of subsequent reactions. The outstanding stability, even after extended storage at room temperature without the use of any cooling agent, makes the QIAcuity Probe PCR Kit ideal for high-throughput reaction setup and plate-stack handling.

The kit works in conjunction with the QIAcuity Digital PCR System (**www.qiagen.com/qiacuity-digital-pcr-system**) and the QIAcuity Nanoplates (**www.qiagen.com/qiacuity-nanoplates-and-accessories**).

For further information please refer to *QIAcuity User Manual* on **www.qiagen.com**.

Solutions for RNA targets

The dPCR Microbial DNA Detection Assay Portfolio contains a number of targets derived from a viral RNA that must be translated into cDNA prior to dPCR analysis. This can be done either in a 2-step or 1-step workflow (see Figure 2).

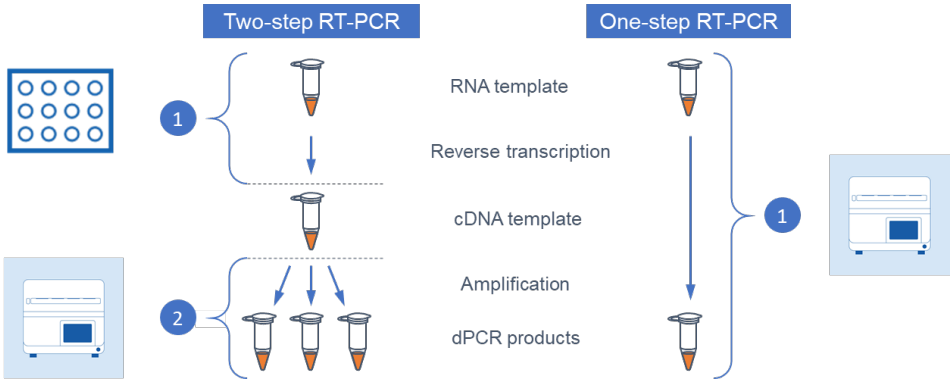

Figure 2. Workflows of 2-step and 1-step RT-PCRs using RNA templates. Target RNA Detection via cDNA synthesis can be done following a 2-step or a 1-step workflow. In the 2-step workflow cDNA synthesis occurs in a separate steps. The resulting cDNA is diluted and used as a template in a second step, the dPCR reaction which is done in the well of the dPCR nanoplate. In the 1-step procedure cDNA synthesis and dPCR amplification are both done in a single well of the dPCR nanoplate.

RNA Preparation

The most important prerequisite for any RNA target analysis experiment is consistent, high quality RNA from every experimental sample. Residual traces of proteins, salts, or other contaminants may degrade the RNA or decrease the efficiency of enzyme activities necessary for optimal RT and real-time PCR performance.

High-quality total RNA for your real-time PCR experiment should be prepared using one of the methods described below, depending on the biological sample. For optimal results, RNA samples should be eluted in RNase-free water. For further details on RNA preparation methods and recommended RNA preparation kits refer to the *QIAcuity User Manual* at **www.qiagen.com**.

Important: Do not use DEPC-treated water.

1-Step Workflow

In the 1-step workflow, both, cDNA synthesis and dPCR amplification occur in the well of the dPCR nanoplate (Figure 2). Because the Mastermix has to contain both RT enzyme and TAQ polymerase 1-step workflows require the use of a mastermix other than the QIAcuity Probe Mastermix.

The QIAcuity One-Step Advanced Probe Kit is the recommended 1-step solution for the quantification of RNA and DNA targets with hydrolysis probes in a singleplex or multiplex (up to 5 targets). It is optimized for nanoplate digital PCR on the QIAcuity and prevents uncontrolled RT activity.

2-Step Workflow

In the 2-step workflow cDNA synthesis and dPCR amplification are separated. To this end the extracted RNA has to be reverse-transcribed into cDNA in a separate reaction prior usage in dPCR. After reverse transcription, the resulting cDNA is diluted and used as a template in a second step, the dPCR reaction which is done in the wells of the dPCR nanoplate (Figure 2).

cDNA synthesis

For cDNA synthesis the use of the QuantiTect Reverse Transcription Kit is recommended. Please follow the instructions provided in the *QuantiTect Reverse Transcription Kit Handbook*.

General considerations

The number of transcripts is calculated using Poisson statistics based upon the total number of positive partitions. In extreme cases, that is:

- Samples with low number of transcripts: expression values might vary from the true expression as a rare transcript could be lost in the dead volume or as the rare transcript might not be correctly represented in the fraction of the original cDNA that was used for quantification.
- Samples with high number of transcripts: with increasing number of transcripts being randomly distributed over the partitions, a partition could contain multiple transcripts. In extreme cases, when all partitions are positive, it will no longer be possible to conduct any quantification.

In case the expression level of your gene of interest is unknown and not expected to be low, we recommend to use cDNA dilutions of 1:10 and 1:100 as input template for dPCR.

Purification of microbial DNA for detection in dPCR

A successful dPCR reaction depends on the purity and integrity of the template, primers, and probes used. For the dPCR Microbial DNA Detection Assays the template is DNA either purified from a biological sample being analyzed or cDNA synthesized from RNA, or a known amount of DNA to be used as a standard or positive control. Primers and probes are DNA oligonucleotides which are typically purchased from a commercial supplier. Because PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, and EDTA than single-step enzyme-catalyzed reactions.

Purification of microbial genomic DNA from highly diverse sample material requires the use of dedicated extraction chemistries that are optimized for handling its specific contaminants. Purity of nucleic acid templates is important for dPCR because contaminants can interfere with fluorescence detection. QIAGEN® offers a complete range of nucleic acid purification systems

that are dedicated for the isolation of bacterial, fungal, and viral DNA and RNA from a variety of biological samples and that provide pure, high-quality templates for PCR and RT-PCR. These include QIAprep® Kits for purification of plasmid DNA, QIAamp®, and DNeasy® Kits for purification of genomic DNA. A selection of purification kits is listed in Table 1. Details about QIAGEN kits for nucleic acid purification can be found at **www.qiagen.com/dna-purification/microbial-dna**.

Table 1. Recommended DNA purification kits by sample type

Sample material	DNA purification kit	Catalog no.
Stool	QIAamp PowerFecal Pro DNA Kit	51804
	QIAsymphony PowerFecal Pro DNA Kit	938036
	RNeasy PowerFecal Pro	78404
	AllPrep PowerFecal Pro DNA/RNA Kit	80254
Blood	QIAamp UCP PurePathogen Blood Kit	50112
	QIAamp DNA Blood Mini Kit	51104
Cervical swab in transport media	QIAamp MinElute Media Kit	57414
Blood culture, bronchoalveolar lavage, carious dentine, cervical swab, isolated bacterial colony, sputum, saliva, swabs	QIAamp UCP Pathogen Mini Kit	50214
	QIAamp UCP DNA Micro Kit	56204
	QIAamp DNA Microbiome Kit	51704
Plasma, serum, cell-free body fluids	QIAamp MinElute Virus Kit	57704
Wastewater	AllPrep PowerViral DNA/RNA Kit	28000-50
	RNeasy PowerFecal Pro Kit	78404

DNA sample digestion

Random template partitioning is essential for accurate quantification in dPCR systems. For the vast majority of QIAcuity dPCR applications, template DNA is uniformly distributed throughout the QIAcuity Nanoplate reaction chambers. In QIAcuity reactions using PCR products, formalin-fixed, paraffin-embedded (FFPE) DNA, circulating cell-free DNA (cfDNA), or complementary DNA (cDNA) as template, a uniform distribution of PCR signal is observed. However, DNA molecules >30 kb are unevenly partitioned, which leads to overquantification of template concentration.

By adding restriction enzymes directly to the QIAcuity reaction mixes, large templates can be fragmented to smaller sizes, which results in even template distribution and accurate quantification. When adding restriction enzymes to reaction mixes, users must be sure that the enzymes do not cut within the amplicon sequence. A summary of recommended enzymes is listed in Table 2.

Table 2. List of recommended enzymes

6-Cutter restriction enzymes		4-Cutter restriction enzymes	
*Eco*RI	0.25 U/µl EcoRI-HF®, NEB® 0.025 U/µl Anza™ 11 EcoRI, TFS	*Alu*I	0.025 U/µl AluI, NEB 0.025 U/µl Anza 44 AluI, TFS
*Pvu*II	0.025 U/µl PvuII, NEB 0.025 U/µl Anza 52 PvuII, TFS	*Cvi*QI	0.025 U/µl CviQI, NEB 0.025 U/µl Csp6I (CviQI), TFS
*Xba*I	0.025 U/µl Anza 12 XbaI, TFS	*Hae*III	0.025 U/µl BsuRI (HaeIII), TFS

NEB, New England Biolabs; TFS, Thermo Fisher Scientific.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Genomic DNA or RNA isolation kit (check Table 1, page 18, for DNA purification kit recommendations)
- Digital PCR instrument; the table on page 3 indicates the appropriate dPCR cyclers.
- QIAcuity dPCR Nanoplates; the table on page 3 indicates the appropriate nanoplates.
- Multichannel pipettor
- Nuclease-free pipette tips and tubes

Important Notes

For accurate and reproducible dPCR results, it is essential to avoid contamination of the assay with foreign DNA, especially PCR products from previously run nanoplates. The most common sources of DNA contamination are the products of previous experiments and highly concentrated template dilutions.

To maintain a working environment free of DNA contamination, we recommend the following precautions:

- Wear gloves throughout the procedure. Use only fresh PCR-grade labware (tips and tubes).
- Use sterile pipette tips with filters.
- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents.
- Physically separate the workspaces used for dPCR setup and post-dPCR processing operations. Decontaminate your dPCR workspace and labware (pipets, tube racks, etc.) with UV light before each new use to render any contaminated DNA ineffective in dPCR through the formation of thymidine dimers or with 10% bleach to chemically inactivate and degrade any DNA.
- Do not open any previously run and stored dPCR nanoplate. Removing the sealer foil from dPCR nanoplate releases dPCR product DNA into the air where it can contaminate the results of future experiments. In the event of contamination ensure that any affected labware and bench surfaces are decontaminated.
- Do not remove the dPCR nanoplate from its protective sealed bag until immediately before use.

Protocol: Digital PCR Using dPCR Microbial DNA Detection Assays in 24-well 26K Nanoplates or 96-well 8.5K Nanoplates

This protocol is optimized for the detection and quantification of specific microbial DNA targets using the dPCR Microbial DNA Detection Assays (cat. no. 250207) with the QIAcuity® Probe PCR Kit (cat. nos. 250101, 250102, 250103) in 24-well 26K nanoplates and 96-well 8.5K nanoplates (cat. nos. 250001, 250011, 250021)

Important points before starting

- Use supplied UCP PCR Water for setting up dPCR reactions.
- Limit the number of times each tube of UCP PCR Water and QIAcuity Probe Mastermix is opened to three times. This will help to prevent contamination.
- It is essential to start with high-quality DNA. For recommended genomic DNA preparation methods, refer to the "Purification of microbial DNA for detection in dPCR" section on page 16.
- dPCR Microbial DNA Detection Assays come as a 20x primer-probe mix lyophilized in a single tube and have to be diluted prior usage. For further dilution and storage information please refer to "Shipping and Storage" section on page 4.
- For best results, all DNA samples should be resuspended in DNase-free water or, alternatively, in DNase-free 10 mM Tris buffer, pH 8.0.
- Pipetting accuracy and precision affects the consistency of results. Be sure that all pipets and instruments have been checked and calibrated according to the manufacturer's recommendations. Also make sure that no bubbles are introduced into the wells of the QIAcuity Nanoplate during pipetting.
- To compensate for any potential environmental contamination, it is required to run at least one No Template Control (NTC) sample for the identification assays.

Things to do before starting

- Determine DNA concentration and purity by preparing dilutions and measuring absorbance in 10 mM Tris, pH 8.0 buffer. For best results, the concentration measured at A_{260} should be greater than 10 ng/µl DNA, the A_{260}/A_{280} ratio should be greater than 1.8.
- Thaw genomic DNA and QIAcuity Probe Mastermix on ice (4°C). After thawing, mix gently by repeated pipetting or quick vortex, then quick spin.
- Dilute 10x Microbial DNA Positive Control to 1x with Microbial DNA-Free Water.

Procedure

1. Thaw the QIAcuity Probe PCR Mastermix, template DNA or cDNA, 20x primer-probe mix, and UCP PCR water. Vigorously mix the QIAcuity Probe PCR Mastermix and the individual solutions. Centrifuge briefly to collect liquids at the bottom of the tubes.
2. Prepare a reaction mix for the number of reactions needed according to Table 3. Due to the hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity instrument.
3. Vortex the reaction mix.
4. Dispense appropriate volumes of the reaction mix, which contains all components except the template, into the wells of a standard PCR plate. Then, add template DNA or cDNA into each well that contains the reaction mix.

   Note: The appropriate amount of reaction mix and template DNA depends on various parameters. Please refer to the *QIAcuity User Manual Extension: Application Guide* for details.
5. **Note**: For 2-step RT-PCR, the volume of the cDNA added (from the undiluted reverse-transcription reaction) should not exceed 15% of the final PCR volume.
6. Transfer the content of each well from the standard PCR plate to the wells of the nanoplate.
7. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.

   Note: For exact sealing procedure, please see the *QIAcuity User Manual*.

Table 3. Reaction setup

Component	Volume/reaction		
	Nanoplate 8.5k (24-well, 96-well)	Nanoplate 26k (24-well)	Final concentration
4x QIAcuity Probe PCR Mastermix	3 µl	10 µl	1x
20x primer-probe mix 1*	0.6 µl†	2 µl†	0.6 µM forward primer 0.6 µM reverse primer 0.2 µM probe
20x primer-probe mix 2, 3, 4, 5* (for multiplex)	0.6 µl†	2 µl†	0.6 µM forward primer 0.6 µM reverse primer 0.2 µM probe
Restriction Enzyme (optional)	Up to 1 µl	Up to 1 µl	0.025-0.25 U/µl
UCP PCR water	Variable	Variable	
Template DNA or cDNA (added at step 4)	Variable‡	Variable‡	
Total reaction volume	**12 µl**	**40 µl**	

* For respective dye recommendation for the probe and available channels on the QIAcuity, please see the *QIAcuity User Manual* or the *QIAcuity User Manual Extension: QIAcuity Application Guide*.

† Volume might vary, depending on concentration of the primer/probe mix used.

‡ Appropriate template amount depends on various parameters. Please see the *QIAcuity User Manual Extension: QIAcuity Application Guide* for details.

8. If a restriction enzyme for DNA digestion has been included in the reaction, leave the plate for 10 min at room temperature.

Thermal cycling conditions

1. Program the cycler of the QIAcuity instrument according to Table 4.

Table 4. Cycling conditions

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
2-step cycling (40 cycles*)		
Denaturation	15 s	95
Combined annealing/extension	60 s	58*

* Temperature during annealing/extension and number of cycles might vary depending on assay type.

2. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

Protocol: Detection of Pathogen RNA Targets using the QIAcuity OneStep Advanced Probe Kit

This protocol is optimized for the quantification of RNA and DNA targets using the QIAcuity One-Step Advanced Probe Kit with hydrolysis probes in a singleplex or multiplex (up to 5 targets) reaction using QIAGEN's QIAcuity instruments for digital PCR.

The QIAcuity One-Step Advanced Probe Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. Under these conditions, the components are stable for 12 months without showing any reduction in performance and quality, unless otherwise indicated on the label.

Important points before starting

- Refer to the *QIAcuity User Manual* and *QIAcuity User Manual Extension* for guidance on assay design and experimental setup for the QIAcuity platform.
- The QIAcuity OneStep Advanced Probe Kit has been specially formulated with a warm-start RT enzyme, allowing users to assemble reactions at room temperature and to run up to four and eight plates in parallel on the QIAcuity Four and QIAcuity Eight instrument, respectively.
- The optional Enhancer GC is recommended for use with all ABI TaqMan assays, amplicons >150 nt in length, GC rich amplicons, and RNA targets containing challenging secondary structures.

Procedure

Reaction mix setup

1. Place the 100x Advanced Reverse Transcription Mix on ice. Thaw the 4x QIAcuity One-Step Advanced Probe Master Mix, template RNA, primers, probes, Enhancer GC, and RNase-Free Water. Vigorously mix the QIAcuity One-Step Advanced Probe Master Mix and the individual solutions. Centrifuge the tubes briefly to settle the liquids.
2. Prepare a master mix according to Table 5 and the desired Nanoplate format.

Table 5. Preparing the QIAcuity One-Step Advanced RT-dPCR reaction mix

Component	Volume/reaction		
	Nanoplate 8.5k (96-well)	Nanoplate 26k (24-well)	Final concentration
4x One-Step Advanced Probe Master Mix	3 µl	10 µl	1x
100x OneStep Advanced RT Mix (Reverse Transcription)	0.12	0.4 µl‡	1x
20x primer–probe mix 1*	0.6 µl‡	2 µl‡	0.6 µM forward primer 0.6 µM reverse primer 0.2 µM probe
20x primer–probe mix 2, 3, 4, 5* (for multiplex)	0.6 µl‡	2 µl‡	0.6 µM forward primer 0.6 µM reverse primer 0.2 µM probe
Enhancer GC† (optional)	1.5 µl	5 µl	
RNase-Free Water	Variable	Variable	
Template RNA (added at step 5)‡	Variable	Variable	
Total reaction volume	**12 µl**	**40 µl**	

* For dye recommendations, see QIAcuity User Manual or the QIAcuity User Manual Extension.

† Enhancer GC is recommended for use with all ABI TaqMan assays, amplicons >150 nt in length, GC rich amplicons, and RNA targets containing challenging secondary structures.

‡ Appropriate template amount depends on various parameters.

3. Vortex the reaction mix well. Dispense appropriate volumes of the reaction mix into the wells of a standard 96-well PCR pre-plate.

   Note: The pre-plate may be assembled at room temperature

4. Add template RNA to wells containing the reaction mix. Thoroughly mix the template RNA with the template RNA by pipetting up and down.

One-step RT-dPCR protocol for all QIAcuity instruments

5. Transfer the contents of each well to the wells of a Nanoplate.
6. Seal the Nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.
7. Place the Nanoplate into the QIAcuity instrument and start the dPCR program.

Table 6. QIAcuity RT-dPCR cycling program

Step	Time	Temperature
Reverse Transcription	40 min	50°C
RT enzyme inactivation	2 min	95°C
2-step cycling (40 cycles)		
Denaturation	5 s	95°C
Combined annealing/extension	30 s	60°C*

* Temperature during annealing/extension and number of cycles might vary depending on assay type.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Weak or no signal with Microbial DNA Positive Control (PMCv2)		
a)	The selected fluorescence channel for dPCR data analysis does not comply with the protocol	For data analysis, select one of the 5 channels, green, yellow, orange, red or crimson depending on the used probe dye.
b)	Incorrect programming of the QIAcuity dPCR instrument	Compare the temperature profile with the protocol. See the cycling conditions in Table 4 or Table 6. Refer to the *QIAcuity User Manual*.
c)	PCR extension time too short	Use the extension time specified in the protocol.
d)	PCR was inhibited	Use the recommended DNA isolation method and closely follow the manufacturer's instructions. QIAGEN offers dedicated sample preparation kits developed to complement dPCR Microbial DNA Detection Assays, and provide a complete and efficient workflow for microbial DNA testing.
e)	Incorrect configuration of the PCR	Ensure that reactions were set up according to the reaction mix preparation tables on Table 3 and Table 5. Repeat the dPCR run, if necessary.
f)	The storage conditions for one or more kit components did not comply with the instructions given in "Shipping and Storage".	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
g)	The dPCR Microbial DNA Detection Assay has expired	Check the storage conditions and the expiration date of the reagents and use a new kit, if necessary.
h)	Insufficient starting template	Increase the amount of template genomic DNA.
i)	Assay template not entailed in PMCv2	Check if assay is covered by the PMCv2 (Table 9). Repeat dPCR run with positive control gDNA or synthetic templates, if necessary.

		Comments and suggestions
Signals present for the negative control template (NTC copies/µl > 0)		
a)	Contamination occurred during PCR setup	Repeat the PCR with new reagents. If possible, seal the PCR array/close the PCR tubes directly after addition of the sample to be tested. Make sure to pipet the positive controls last. Make sure that workspace and instruments are decontaminated at regular intervals.
b)	Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that workspace and instruments are decontaminated at regular intervals.
c)	Pan Bacteria Assays and Pan Fungi Assays may detect residual bacterial and fungal genomic DNA found in the QIAcuity Probe PCR Mastermix.	If the average number of measured copies/µl of the sample > limit of blank + 1.645x the standard deviation of the average measured number of copies/µl of the sample, the sample is positive for the Pan Bacteria or the Pan Fungi assays (further details are provided in Appendix A: Data Analysis).

References

1. Armbruster, D.A., Pry, T. (2008) Limit of Blank, Limit of Detection and Limit of Quantitation. Clin. Biochem. Rev. **29**, 49.
2. Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., and Wittwer, C.T. (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. **55**, 611.
3. Burd, E.M. (2010) Validation of laboratory-developed molecular assays for infectious diseases. Clin. Microbiol. Rev**. 23,** 550**.**

Appendix A: Data Analysis

Principle for dPCR Microbial DNA Detection Assays Data Analysis

In digital PCR, detection and quantification of target DNA molecules is based on copies/µl values obtained from individual PCR reactions in nanoscale volumes called partitions. Individual partitions are generated by splitting a PCR reaction over which the target molecules are randomly distributed. After partitioning, some partitions will contain no copy of the target molecule, some will contain one copy of the target molecule, and some others will contain more than one copy of the target molecule. Since the target molecules are randomly distributed, the Poisson distribution can be used to calculate the average number of copies of the target molecule per partition. The total number of copies of the target molecule in all valid partitions of a well is calculated by multiplying the average number of copies of the target molecule per partition by the number of valid partitions. The QIAcuity Software Suite calculates the target DNA molecule concentration in the reaction in copies/µl by referring to the known volume of a valid partition.

For dPCR Microbial DNA Detection Assays, there are two data analysis methods depending on the application. For relative profiling or comparing two different populations (i.e. healthy versus disease, time 0 versus time 30 days), the normalization method is recommended. For identification of microbial species or microbial genes, the NTC method is used. The NTC method allows for higher sensitivity of detection by measuring the background of the assay. For both methods, the copies/µl value for a dPCR assay is positively correlated with the abundance of the microbial species or gene in the sample.

Microbial dPCR Profiling Assays (normalization method)

The normalization method is used for the relative profiling or comparison between two populations. To account for different starting amounts of DNA in a sample, separate reference assays are used to normalize the sample input. Depending on the type of assay and application, total bacterial or fungal genomic DNA in combination with the Pan Bacteria and

Pan Fungi assays can be used for normalization against the bacterial background of the sample. Alternatively, any established assay, for example targeting the host genomic DNA in the sample, can be used.

To calculate the normalized copies/µl values of each sample, the copies/µl values of the replicates for each reference and target assay are averaged. The normalized copy/µl value of the target molecule is obtained by dividing the average copy/µl value of the target molecule by the average copy/µl value of the reference assay. With the normalized copies/µl values the fold increase or decrease in abundance is calculated based on the formula:

Foldchange = Log_2(normalized cp/µl^{Sample1}/ normalized cp/µl^{Sample2})

As an example for the calculation of abundance fold changes between samples using reference genes for normalization Table 7 depicts dPCR analysis results for two samples analyzed in a duplex reaction with a target of interest-specific assay and a reference assay. Each Sample was analysed in triplicates.

Sample1:

Average cp/µl target Sample 1 = (68+73+69)/3=70 cp/µl

Average cp/µl reference Sample 1 = (172+165+164)/3=167 cp/µl

Normalized abundance target Sample 1 = 70/167 = 0.419

Sample 2:

Average cp/µl target Sample 2 = (111+105+114)/3=110 cp/µl

Average cp/µl reference Sample 2 = (79+84+86)/3=83 cp/µl

Normalized abundance target Sample 2 = 110/83 = 1.325

Foldchange in Sample 2 compared to Sample 1:

$FC^{Sample2}$=Log_2(1.325/0.419)=1.661

Table 7. Example dPCR data for 2 samples analyzed with a target and a reference assay.

Nanoplate Well	Sample/NTC/Control	Reaction Mix	Channel	Concentration (copies/µl)
A1	S1-replicate1	target assay	GREEN	68
A1	S1-replicate1	ref assay	YELLOW	172
A2	S1-replicate2	target assay	GREEN	73
A2	S1-replicate2	ref assay	YELLOW	165
A3	S1-replicate3	target assay	GREEN	69
A3	S1-replicate3	ref assay	YELLOW	164
B1	S1-replicate1	target assay	GREEN	111
B1	S1-replicate1	ref assay	YELLOW	79
B2	S1-replicate2	target assay	GREEN	105
B2	S1-replicate2	ref assay	YELLOW	84
B3	S1-replicate3	target assay	GREEN	114
B3	S1-replicate3	ref assay	YELLOW	86

Second level analyses of foldchanges with abundances normalized against reference assays can be done in the QIAcuity Software Suite using the Gene Expression Plugin. Therein, one can select single and multiple reference genes for normalization, as well as analyze single plex and multiplex approaches. For further details refer to the gene expression chapter in the *QIAcuity User Manual Extension: QIAcuity Application Guide* (**www.qiagen.com/HB-2839**).

Microbial dPCR Identification Assays (NTC method)

The NTC method allows for higher sensitivity of detection by measuring the background of the assay expressed as the limit of blank (LOB). The assay background and/or low levels of contamination due to environmental factors can be corrected for by performing a dPCR reaction using No Template Control (NTC) as the sample. NTC is used to establish a threshold for the number of positive partitions above which a target in the sample can be considered as positively detected. This threshold is called the limit of detection (LOD) and can vary between assays and sample types. As an example, Table 8 shows the number of detected copies/µl in 4 NTC and 4 Sample replicates. The LOD is calculated as follows:

Average number of measured copies/µl in NTC ($mean^{NTC}$) = (0+0.055+0)/3=0.018

Standard deviation of measured copies/µl in NTC (SD^{NTC}) = 0.026

Limit of Blank (LOB) = $mean^{NTC}$ +1.645*(SD^{NTC}) = 0.018+1.645*0.026 = 0.061

Average number of measured copies/µl in Sample ($mean^{Sample}$) = (0.268+0.386+0.377)/3 = 0.344

Standard deviation of measured copies/µl in Sample (SD^{Sample}) = 0.054

Limit of Detection (LOD) = LOB +1.645*(SD^{Sample}) = 0.061+1.645*0.054 = 0.149 copies/µl

LOD in total copies per reaction (8.5K nanoplate) = LOD * 12 = 0.149*12 = 1.79 copies

LOD in total copies per reaction (26K nanoplate) = LOD * 40 = 0.149*40 = 5.96 copies

For this particular example, the target can be considered as present as the on-average measured copies/µl value ($mean^{Sample}$) is > 0.149

For cases of ($mean^{Sample}$) < LOD, the sample should be considered absent for the target.

Table 8. Example of detected positive partitions for 3 NTC and 3 Sample replicates.

Replicate	Measured Copies/µl
NTC Replicate 1	0
NTC Replicate 2	0.055
NTC Replicate 3	0
Sample Replicate 1	0.268
Sample Replicate 2	0.386
Sample Replicate 3	0.377

For further details on the Limit of Detection (LOD), the Limit of Blank (LOB), please refer to the literatures listed in the “References” section.

Appendix B: Microbial DNA Positive Control (PMCv2)

The Microbial DNA Positive Control can be used to ensure that the experimental conditions and PCR setup are correct. It is a mixture of synthetic oligonucleotides that is supplied at a 10x concentration. The synthetic oligonucleotides are targets for the primer/hydrolysis probes. To use the Microbial DNA Positive Control, dilute to 1x with EB buffer or Tris·Cl as follows:

- 5 µl of 10x Microbial DNA Positive Control + 45 µl EB buffer or Tris·Cl.

  Note: 1x Microbial DNA Positive Control can be stored at –30 to –15°C. Avoid repeated freeze/thaw cycles of 1x Microbial DNA Positive Control.

1 µl of the 1x Microbial DNA Positive Control is contains approximately 2000 target copies. The recommended amount to use when setting up an assay is as follows:

- 8.5k 96-well nanoplate: 0.6 µl of 1x Microbial DNA Positive Control per 12 µl reaction.
- 26k 24-well nanoplate: 2 µl of 1x Microbial DNA Positive Control per 40 µl reaction.

Under proper assay setup and PCR conditions, the Microbial DNA Positive Control will yield ~100 copies/µl reaction.

Portfolio Coverage

The Microbial DNA Positive Control PMCv2 (cat. no. 338135) does not contain templates for all assays of the dPCR Microbial DNA Assay portfolio. Table 9 lists all assays currently not covered by the Microbial DNA Positive Control.

Table 9. Assays not entailed in the Microbial DNA Positive Control PMCv2.

GeneGlobe cat. no.	Assay name	Target region	Taxonomy/target type
DMA00346	Pan Aspergillus/Candida	28S ribosomal RNA gene	Fungi
DMA00347	Pan Bacteria 1	16S ribosomal RNA gene	Bacteria
DMA00348	Pan Bacteria 3	16S ribosomal RNA gene	Bacteria
DMA00404	Blautia coccoides	16S ribosomal RNA gene	Bacteria
DMA00413	Mobiluncus spp.	16S ribosomal RNA gene	Bacteria
DMA00476	Sarocladium strictum	28S ribosomal RNA gene	Fungi
DMA00477	Aspergillus terreus (2)	Cis-aconitate decarboxylase (cadA) gene	Fungi
DMA00479	Fusarium solani	Beta tubulin gene	Fungi
DMA00480	Neoscytalidium dimidiatum	Translation elongation factor 1 alpha gene	Fungi
DMA00481	Alternaria alternata	Environmental Microbes; Infectious Diseases	Fungi
DMA00482	Aspergillus versicolor	Mitochondrial small ribosomal RNA gene	Fungi
DMA00483	Curvularia lunata	Glyceraldehyde 3-phosphate dehydrogenase gene	Fungi
DMA00484	Scopulariopsis brevicaulis	18S ribosomal RNA gene	Fungi
DMA00485	Human astrovirus1	ORF1b polyprotein gene	Viruses
DMA00486	Sapovirus sp.	Polyprotein gene	Viruses
DMA00487	Enterovirus	Polyprotein gene	Viruses
DMA00488	Human rotavirus A	Outer capsid protein (VP4) gene	Viruses
DMA00489	Aspergillus terreus (1)	Internal Transcribed Spacer ribosomal RNA	Fungi
DMA00490	Oxalobacter formigenes	16S ribosomal RNA gene	Bacteria
DMA00491	Methanobrevibacter smithii	RNA polymerase subunit B-like (rpoB) gene	Bacteria
DMA00584	fosA (Pseudomonas sp.)	Bacterial FosA family fosfomycin resistance glutathione transferase gene	Antibiotic Resistance
DMA00589	TEM	Bacterial class A broad-spectrum beta-lactamase TEM-1 gene	Antibiotic Resistance

Table continues on next page

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GeneGlobe cat. no.	Assay name	Target region	Taxonomy/target type
DMA00595	aatA	Bacterial outer membrane protein (aatA) gene	Virulence
DMA00694	Cronobacter spp.	Macromolecular synthesis (MMS) operon	Bacteria
DMA00695	Listeria spp.	Invasion associated protein p60 (iap) gene	Bacteria
DMA00696	Escherichia coli (stx1)	Shiga toxin 1 subunit A (stx1A) gene	Bacteria
DMA00697	Escherichia coli (stx1)	Shiga toxin 2 subunit A (stx2A) gene	Bacteria
DMA00698	Escherichia coli (eae)	E. coli attaching and effacing (eae) gene	Bacteria
DMA00699	Escherichia coli (rfbE)	GDP-perosamine synthase RFbE/PerA gene	Bacteria
DMA00704	Salmonella spp. (2)	Invasion protein (invA) gene	Bacteria
DMA00705	Legionella spp.	16S ribosomal RNA gene	Bacteria
DMA00706	Legionella pneumophila (2)	Macrophage infectivity potentiator (mip) surface protein gene	Bacteria
DMA00707	Listeria grayi	Invasion associated protein p60 (iap) gene	Bacteria
DMA00710	Human corona virus SARS-CoV-2 N1	Conserved region 1 (N1) ribonucleoprotein gene	Viruses
DMA00711	Human corona virus SARS-CoV-2 N2	Conserved region 2 (N2) ribonucleoprotein gene	Viruses
DMA00712	Influenza A (2)	Matrix protein 1 gene (M1)	Viruses
DMA00713	Influenza A (3)	Matrix protein 1 gene (M1)	Viruses
DMA00714	Influenza B (2)	Nuclear export protein gene (NEP)	Viruses
DMA00715	Respiratory syncytial virus (2)	Matrix protein gene (M)	Viruses

To determine if the assays, that are not present in the PMCv2 do work, use samples that are known to have bacterial and fungal genomic DNA (i.e., isolated bacterial or fungal colony, metagenomic samples, etc.) or viral target DNA as a positive control.

Ordering Information

Product	Contents	Cat. no.
dPCR Microbial DNA Detection Assays	One tube with lyophilized dPCR Microbial DNA Detection Assay	250207
Accessories		
QIAcuity Probe PCR Kit (1 ml)	1 tube of 1.00 ml 4x concentrated QIAcuity Probe Mastermix, 2 tubes of 1.90 ml water each.	250101
QIAcuity Probe PCR Kit (5 ml)	5 tubes of 1.00 ml 4x concentrated QIAcuity Probe Mastermix each, 8 tubes of 1.90 ml water each.	250102
QIAcuity Probe PCR Kit (25 ml)	5 tubes of 5.00 ml 4x concentrated QIAcuity Probe Mastermix each, 4 tubes of 20 ml water each.	250103
Microbial DNA-Free Water	12 tubes of 1.35 ml each	338132
10x Microbial DNA Positive Control V2 (PMCv2)	One tube of 50 µl mix of synthetic DNA templates	338135
Nanoplate 24-well 26K	Microfluidic digital PCR plates for 24 samples with up to 26,000 partitions each	250001 250002
Nanoplate 24-well 8.5K	Microfluidic digital PCR plates for 24 samples with up to 8,500 partitions each	250011
Nanoplate 96-well 8.5K	Microfluidic digital PCR plates for 96 samples with up to 8,500 partitions each	250021
Nanoplate Seals	11 top seals for the nanoplates	250099

Product	Contents	Cat. no.
QIAcuity 1 2-channel	One-plate digital PCR instrument for detecting up to 2 fluorescent dyes	911001
QIAcuity 1 5-channel	One-plate digital PCR instrument for detecting up to 5 fluorescent dyes	911021
QIAcuity 4 5-channel	Four-plate digital PCR instrument for detecting up to 5 fluorescent dyes	911042
QIAcuity 8 5-channel	Eight-plate digital PCR instrument for detecting up to 5 fluorescent dyes	911052
Related products		
QIAcuity One-Step Viral RT-PCR Kit	4x 1.3 ml One-Step Viral RT-PCR Master Mix (4x), 2x 100 µl Multiplex Reverse Transcription Mix (100x) and 8x 1.9 ml RNase-Free Water; for 500 reactions in Nanoplate 26K and 1666 reactions in Nanoplate 8.5K	1123145
QIAcuity One-Step Advanced Probe PCR Kit (1 ml)	1 ml OneStep Advanced Probe Master Mix (4x), 45 µl OneStep RT Mix (100x), 1 ml Enhancer GC, 20 µl QN Internal Control RNA, 2 x 1.9 ml RNase-free water; for 100 reactions in Nanoplate 26K and 333 reactions in Nanoplate 8.5K	250131
QIAcuity One-Step Advanced Probe PCR Kit (5 ml)	5 x 1 ml OneStep Advanced Probe Master Mix (4x), 5 x 45 µl OneStep RT Mix (100x), 5 x 1 ml Enhancer GC, 1 x 20 µl QN Internal Control RNA, 8 x 1.9 ml RNase-free water; for 500 reactions in Nanoplate 26K and 1666 reactions in Nanoplate 8.5K	250132

Product	Contents	Cat. no.
QIAamp Fast DNA Stool Mini Kit	For 50 preps, includes QIAamp Mini Spin Columns, QIAGEN Proteinase K, InhibitEX Buffer, wash and elution buffers, Collection Tubes (2 ml)	51604
QIAamp UCP PurePathogen Blood Kit	For 10 preps, includes: QIAamp UCP Mini columns, QIAGEN Proteinase K, Tube Extenders (20 ml), Buffers, VacConnectors, Pathogen Lysis Tubes, and Collection Tubes (2 ml)	50112
QIAamp MinElute Media Kit	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Proteinase K, Carrier RNA, Buffers, Extension Tubes (3 ml), Collection Tubes (1.5 ml)	57414
QIAamp UCP Pathogen Mini Kit	50 QIAamp UCP Mini Columns, Collection Tubes (2 ml), Tube Extenders (20 ml), Elution Tubes, VacConnectors, Buffers, and Proteinase K	50214
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Furthermore, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training, and preventive subscription. Contact your local sales representative to learn about your options.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
04/2022	Initial revision

Notes

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HB-3059-001 04/2022