Product Profile

dPCR Microbial DNA Detection Assays

Detect microbial targets – bacterial, fungal, parasitic, viral, antibiotic resistance and virulence factor genes – using digital PCR

The dPCR Microbial DNA Detection Assays are hydrolysis probe-based assays for precise and sensitive identification of a wide range of microbial targets using digital PCR (dPCR). They consist of a primer pair and a hydrolysis probe with a configurable dye (fluorophore) that allows multiplex detection of up to five targets per reaction.

The assays are intended for use with the QIAcuity® Probe PCR Kit (for DNA targets) or the QIAcuity OneStep Advanced Probe Kit (for RNA or RNA+DNA targets) on the QIAcuity Digital PCR System.

- Identify more than 680 microbial targets
- Detect up to five targets per reaction using five fluorophores – FAM™, HEX™, TAMRA, ROX™, Cy5®
- Follow a simple and fast dPCR workflow on the QIAcuity Digital PCR System
- Combine microbial DNA and viral RNA detection in one reaction

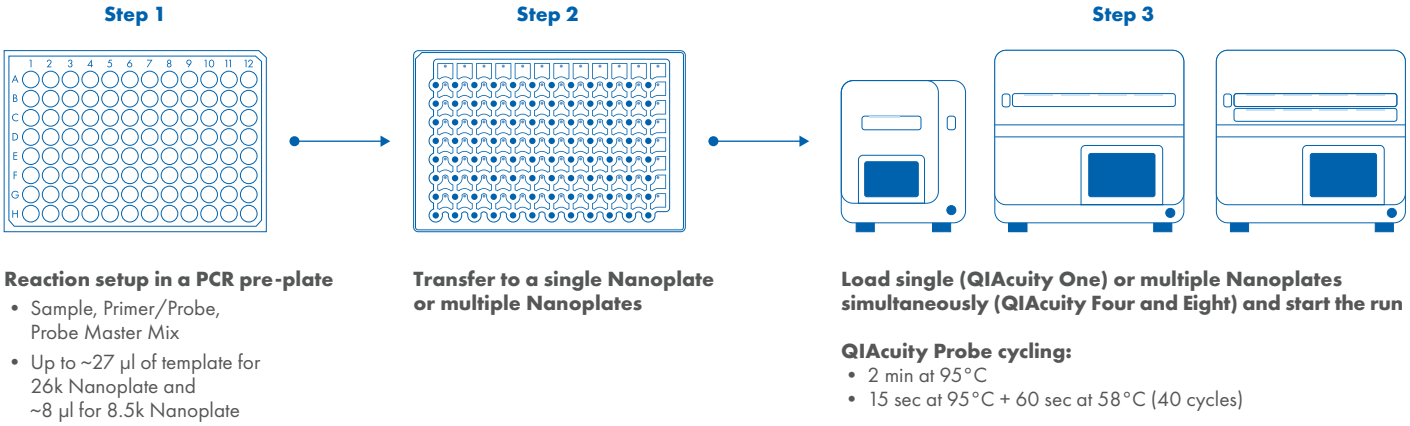


Figure 1. Microbial dPCR in about two hours with minimal hands-on time.
This example shows a Nanoplate 8.5k 96-well. The recommended Nanoplate for higher sensitivity is Nanoplate 26k 24-well.

Explore the virtual workflow: **www.qiagen.com/applications/digital-pcr/workflow/qiacuity-demo**

Highly sensitive detection of over 680 microbial targets

dPCR Microbial DNA Detection Assays can be used to test the presence of over 680 bacterial, fungal, parasitic, viral, antibiotic resistance or virulence factor genes (Table 1). The high sensitivity of the QIAcuity Digital PCR System allows detection of microorganisms or their genes present at extremely low levels such as in metagenomic samples, isolated colonies or other challenging sample types. A streamlined workflow gets you to results in just two hours with minimal hands-on time (Figure 1).

Table 1. Targets covered by the dPCR Microbial DNA Detection Assays

Bacterial targets	Fungal targets	Viral targets	Virulence and antibiotic resistance targets
387 targets	35 targets	46 targets	202 targets
Examples: *E. coli, Salmonella, Yersinia, Legionella, Vibrio, Clostridia, Streptococcus, Mycobacteria, Mycoplasma* species	Examples: *Aspergillus, Candida, Trichophyton, Fusarium, Microsporum* species	Examples: SARS-CoV-2 (N1, N2), Influenza A&B, RSV, Norovirus GI & II	Examples: Beta lactamases, MDR efflux pumps, fluoroquinolone resistances, toxins, components of microbial secretion systems, surface proteins

For a detailed target list, see the Technical Information: **www.qiagen.com/TI_dPCR-microbial-detection**

Accurate quantification of microbial targets

To demonstrate accurate quantification of microbial targets using the dPCR Microbial DNA Detection Assays on the QIAcuity Digital PCR System, we quantified the NIST reference standard 8376 (**https://www-s.nist.gov/m-srmors/certificates/8376.pdf**) using genomic DNA (gDNA) from *Shigella sonnei* as input.

The measured concentration (copies/µl) of the target gene closely matches the expected concentration, as shown in Figure 2.

Figure 3 illustrates a four-log dynamic range of linear quantification of two bacterial targets – *Enterococcus faecalis* and *Shigella sonnei* – down to 0.4 copies/µl.

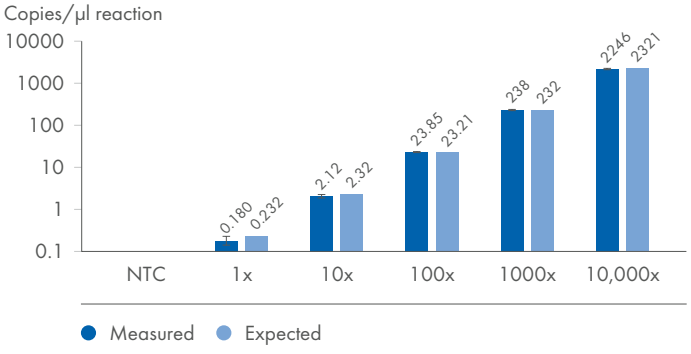
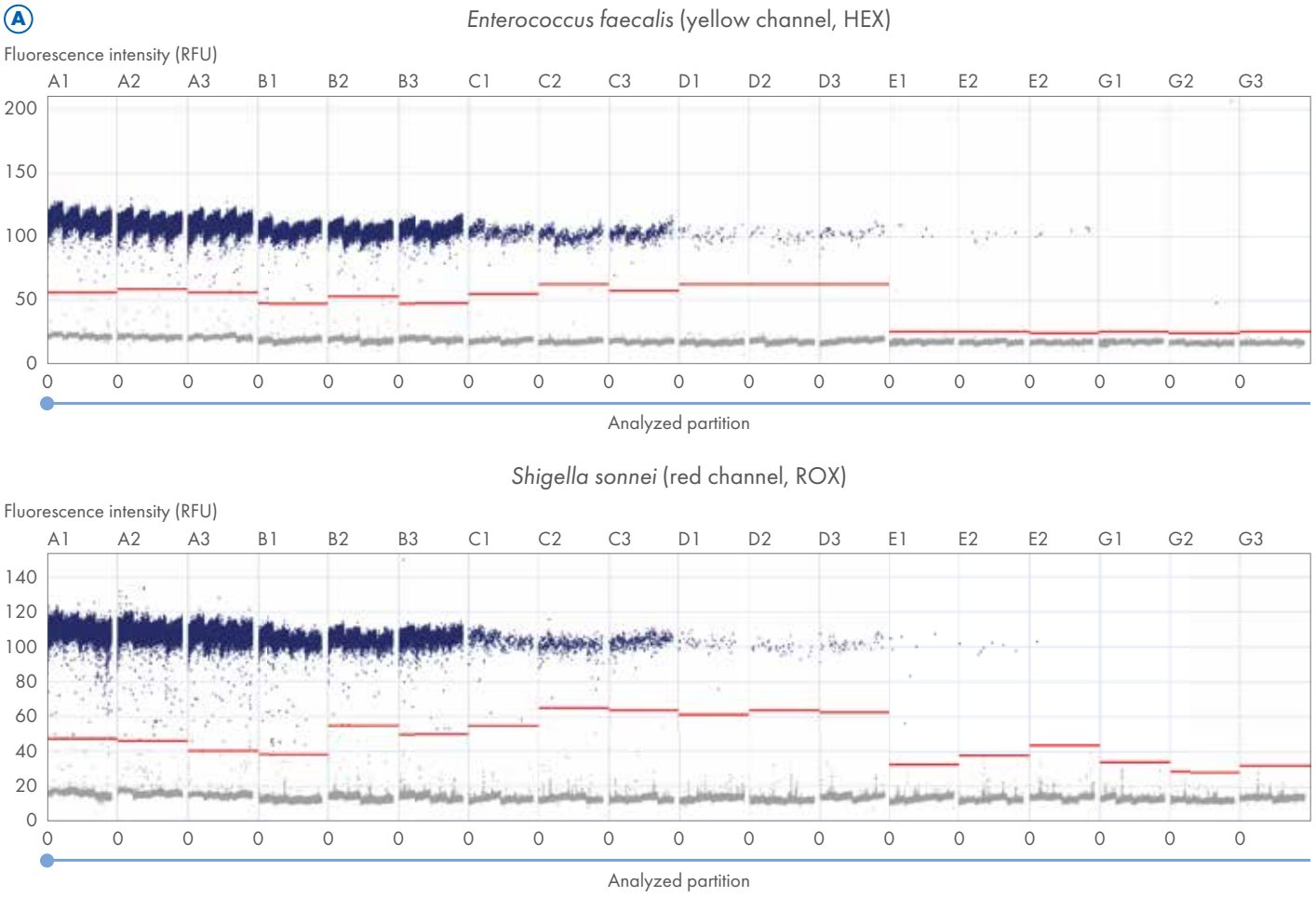


Figure 2. Accurate quantification of a reference standard. The NIST reference standard 8376 was quantified using gDNA from *S. sonnei* as input. The dPCR assay that targets a single copy gene of S. *sonnei* revealed accurate absolute quantification of the expected copy numbers in all dilutions of the reference DNA template (1x – 10,000x). Dark blue bars represent the mean measured copies/µl values with error bars from three replicates each run using a 26k 24-well Nanoplate on the QIAcuity Digital PCR System.



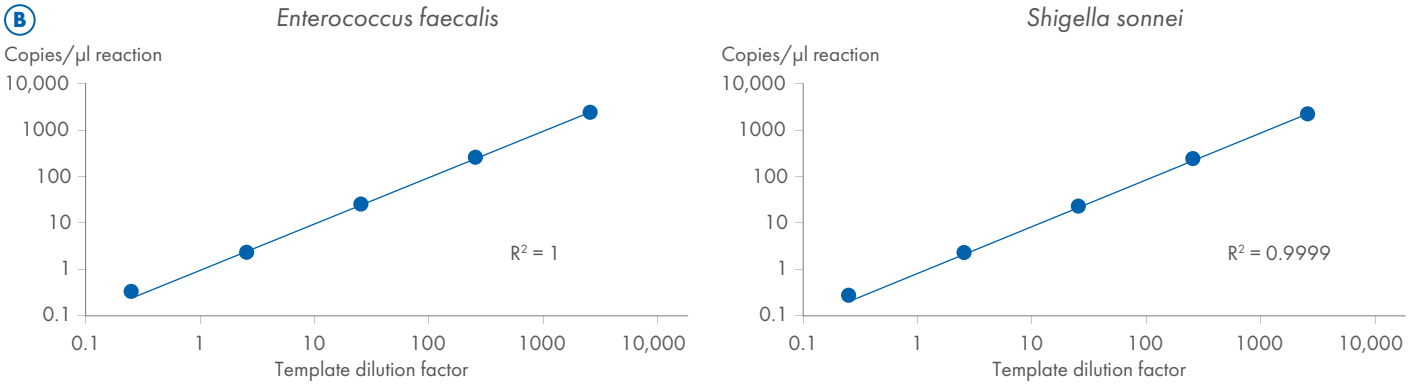


Figure 3. Four-log dynamic range of linear quantification of target species.
A 1D scatter plots of replicates (three each) of five template dilutions and a no template control (NTC) for *E. faecalis* and *S. sonnei* using gDNA as input template.
B Respective plots of the mean measured copies/µl values against the dilution factor of the input template. R^2 values indicate a strong positive linear relationship between the measured copies/µl values and the dilution factor of the input template. dPCR was performed using 26k 24-well Nanoplates on the QIAcuity Digital PCR System.

Precise and specific quantification in singleplex and multiplex

The choice of multiple fluorescent dye combinations enables quantification of up to five targets per reaction. This saves time, money and precious sample material. Experiments can be set up using fewer (than usual) wells in a Nanoplate, allowing higher sample throughput. Furthermore, you can make your own multiplex assay by ordering single assays with different dyes (FAM, HEX, TAMRA, ROX, Cy5). Several wet-lab tested and validated 5-plex bundles are available; other combinations need to be verified by the customer. The performance of the dPCR Microbial DNA Detection Assays are comparable in singleplex and multiplex (Figure 4). Figure 5 shows the strong signal to noise separation observed in multiplex assays. Figure 6 illustrates multiplex detection of five water microbial pathogens in a single run.

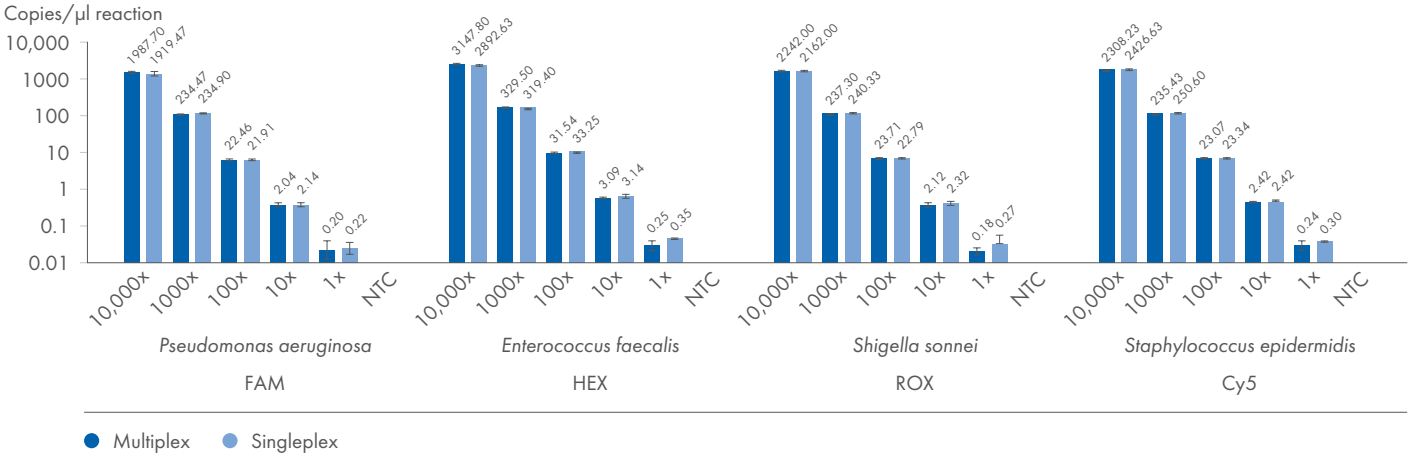


Figure 4. Singleplex versus multiplex setup quantifying four different bacterial targets.
Four assays were run in singleplex and 4-plex reactions using the same template gDNA material. In both setups, the same concentrations were observed for template dilutions between 1x and 10,000x. dPCR, with three replicates per condition, was performed using 26k 24-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.

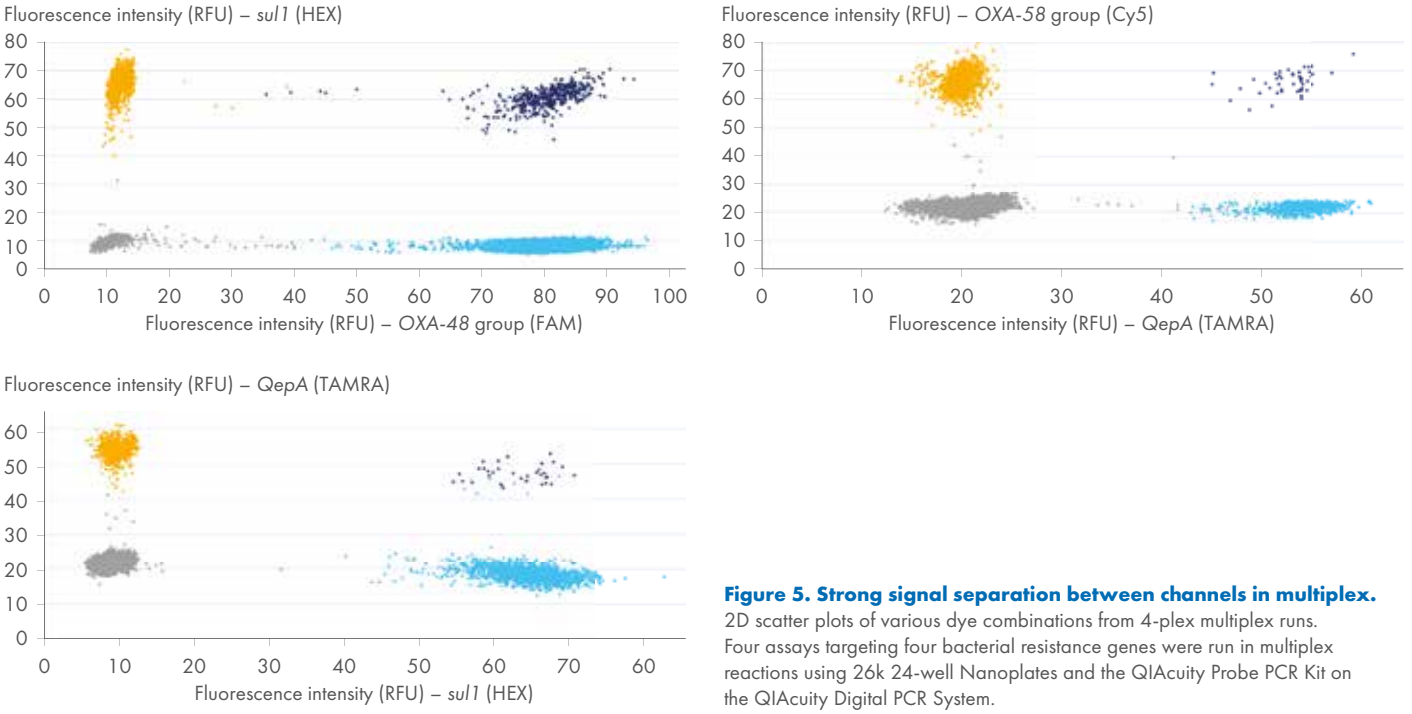


Figure 5. Strong signal separation between channels in multiplex.
2D scatter plots of various dye combinations from 4-plex multiplex runs. Four assays targeting four bacterial resistance genes were run in multiplex reactions using 26k 24-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.

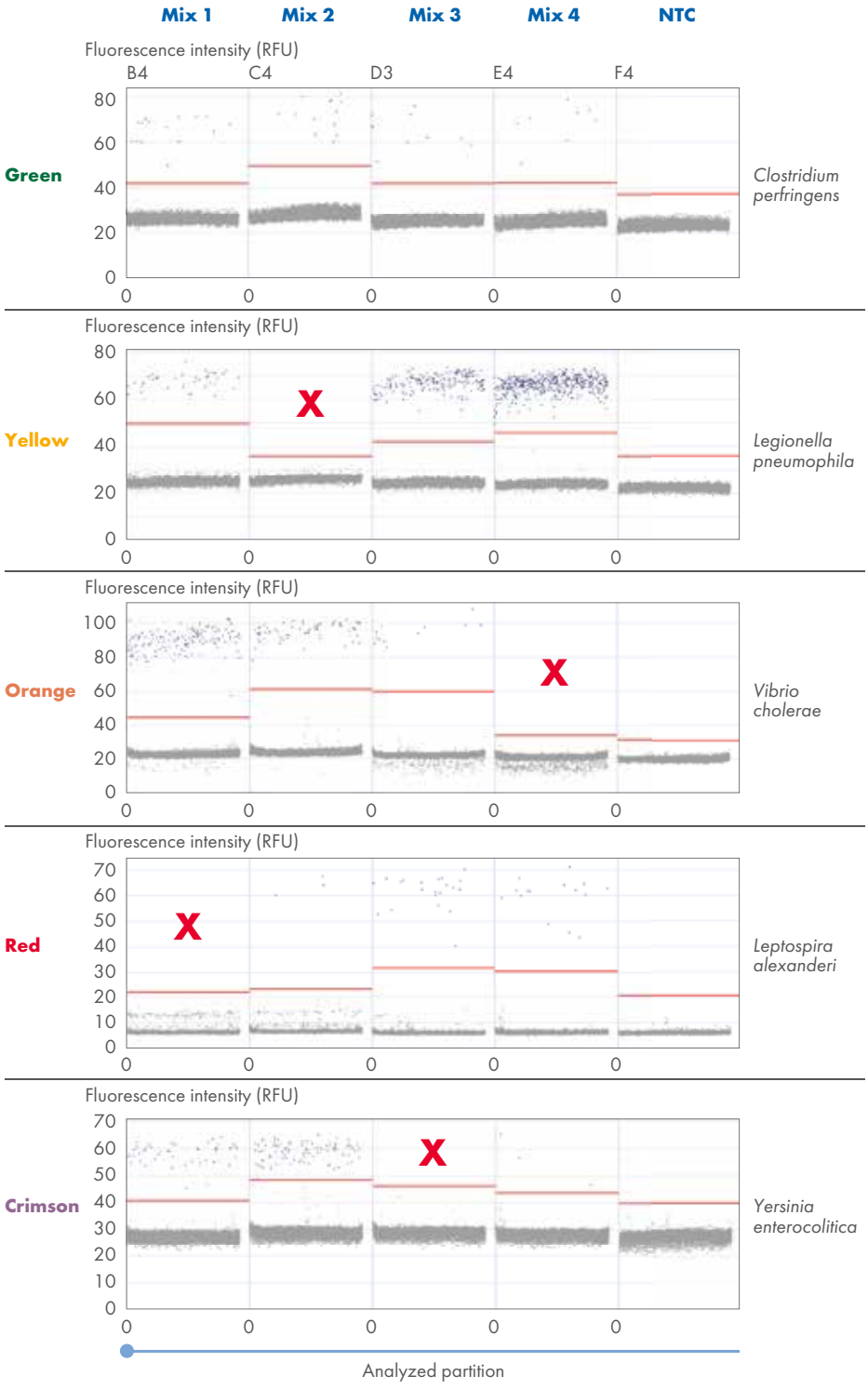


Figure 6. Water microbial pathogen detection in 5-plex.
1D scatter plot showing five reactions with four different template mixes and a no template control (NTC). Each gDNA mix contained different amounts of gDNAs from four out of five target organisms (red crosses mark the missing organism in a particular mix); mix 1 lacks *Leptospira* template; mix 2 lacks *Legionella* template; mix 3 lacks *Yersinia* template; mix 4 lacks *Vibrio* template. Each target DNA was quantified in the 5-plex reaction. dPCR, with three replicates per condition, was run using 8.5k 24-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.

Detect viral RNA and microbial DNA together in a multiplex reaction

dPCR Microbial DNA Detection Assays, together with the new QIAcuity OneStep Advanced Probe Kit, enables detection of RNA targets or a combination of RNA and DNA targets in one multiplex reaction. This is particularly useful for the simultaneous detection of DNA and RNA viruses. Figure 7 illustrates simultaneous detection of the SARS-CoV-2 (N1) RNA target and four bacterial targets in multiplex. Viral target-only combinations are also possible, for example, multiplex detection of SARS-CoV-2 and Influenza or Norovirus (data not shown).

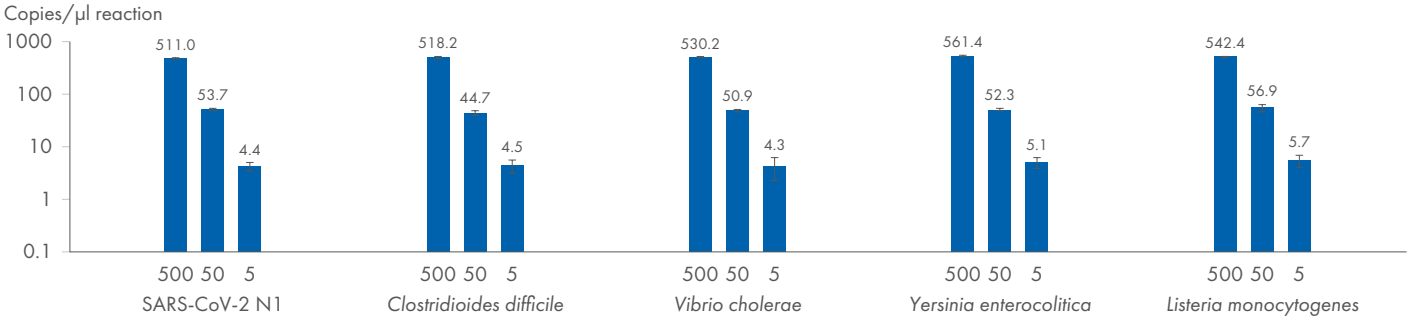


Figure 7. Multiplex detection of RNA and DNA targets.
A mixture of four bacterial gDNAs (*Clostridioides difficile, Vibrio cholerae, Yersinia enterocolitica, Listeria monocytogenes*) and SARS-CoV-2 RNA was used as input. dPCR, with three replicates per condition, was run using 8.5k 96-well Nanoplates and the QIAcuity OneStep Advanced Probe Kit on the QIAcuity Digital PCR System. Three template dilutions with 500, 50 and 5 copies/µl were used. Barchart shows the mean measured concentrations (copies/µl) of three replicates each for each of the five targets.

Ordering Information

Product	Contents	Cat. no.
dPCR Microbial DNA Detection Assays	One tube Microbial DNA dPCR Assay, 200 x 40 µl reactions in Nanoplate 26k, 666 x 12 µl reactions for Nanoplate 8.5k	250207 (Respective Assay Cat. No. to be specified in GeneGlobe®)
QIAcuity Probe PCR Kit (1ml, 5 ml)	1 ml or 5 ml Master Mix for the QIAcuity dPCR instrument; water	250101, 250102
QIAcuity OneStep Advanced Probe Kit (1 ml, 5 ml)	1 ml or 5 ml OneStep Master Mix for the QIAcuity dPCR instrument, RT Mix, Internal Control RNA, Enhancer GC, Water	250131, 250132

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.

For configuring and ordering the assays, please visit:
geneglobe.qiagen.com/products/analysis-type/analysis-type-dpcr