IT STARTS WITH A DROPLET AND ENDS IN DISCOVERY.
Droplet Digital PCR (ddPCR™) is Bio-Rad’s unique digital PCR technology. With unrivaled precision, ddPCR provides absolute quantification of target DNA or RNA molecules without the use of standard curves. ddPCR addresses the lack of scalable and practical technologies for digital PCR implementation. The new QX200 ddPCR system puts this powerful technology in your hands, ready to unveil new worlds of research at previously unattainable levels.

PCR becomes “digital” through sample partitioning and the subsequent statistical analysis of target detection across the partitions. The QX200 system uses advanced microfluidics technology to achieve partitioning on a massive scale, generating 20,000 highly uniform nanoliter-sized droplets per sample. For even higher sensitivity, it is possible to combine wells and analyze millions of droplets for a single sample.

Simple and reliable, Droplet Digital PCR technology has already led to several research breakthroughs in fields such as cancer biomarker discovery, infectious diseases, genomic alterations, and gene expression. This research is only the beginning. What discoveries will you make using Droplet Digital PCR?
Droplet Digital PCR has a simple, user-friendly experiment setup designed to process eight samples at a time. The process easily scales up to run a 96-sample experiment in 5 hours with minimal hands-on time. When higher throughput is required, multiple 96-sample experiments can be run in a day.

Bio-Rad’s QX200 ddPCR system combines water-oil emulsion droplet technology with microfluidics. The QX200 system consists of two instruments — a droplet generator and a droplet reader — and associated consumables. The droplet generator partitions each sample into 20,000 uniform nanoliter-sized droplets in which nucleic acid molecules are distributed in a random fashion. Each droplet serves to partition the reactions. Droplets are transferred to a 96-well PCR plate and PCR is performed to end point in a thermal cycler. The droplets stream single file through the reader for fluorescence analysis. Positive droplets, which contain at least one copy of the target DNA or RNA molecule, exhibit increased fluorescence compared to negative droplets. The fraction of PCR-positive droplets enables the target to be quantified according to the Poisson distribution.

The QX200 system works with both hydrolysis probes and EvaGreen fluorescence detection chemistries, and its flexible design allows for high throughput and ultra-sensitive detection.

**KEY BENEFITS**

- Achieve absolute quantification without the use of a standard curve
- Design scalable assays for high sensitivity or high throughput
- Expand applications using flexible ddPCR chemistry — EvaGreen or probes
prepare ddPCR reaction mix

- Combine DNA sample, primers, and/or probes with one of Bio-Rad’s ddPCR supermixes
- Fully validated PrimePCR ddPCR assays can be used

Generate droplets

- Load the ddPCR reaction mix into the wells of a droplet generator cartridge
- 8 x 20,000 droplets are generated from each run in the QX200 droplet generator
- Target DNA (●) and background DNA (●) are randomly distributed in droplets

Perform PCR with EvaGreen or hydrolysis probes

- Transfer the droplets to a 96-well PCR plate and seal the plate
- Run the PCR protocol

Read and analyze results

- After PCR, load the 96-well PCR plate into the QX200 droplet reader
- Positive and negative droplets in each sample are read
- Analyze concentrations with QuantaSoft™ software

For more information, visit www.bio-rad.com/QX200.
WHAT'S IN A DROPLET?

QUANTIFYING BIOMARKERS FOR CANCER

The level of sensitivity offered by the QX200 ddPCR system in quantifying cancer biomarkers overcomes the limitations posed by other methods. Using Droplet Digital PCR technology, researchers are now able to observe finer quantitative distinctions among mutations, better identifying their potential roles in cancer.

Cancer-associated mutations often evade detection due to their low concentrations relative to the background of wild-type DNA in a given sample. With its high sensitivity, the QX200 system can easily scale to quantify target concentrations as low as one out of 1,000,000 (0.0001%) total copies. What was previously undetectable with other methods can now be quantified with Droplet Digital PCR.

SUCCESS STORY

Detecting T790M mutation in epidermal growth factor receptor (EGFR), an important therapeutic target in some lung cancers, allows for a better understanding of resistance to tyrosine kinase inhibitor therapies. However, other techniques lacked the sensitivity to reliably identify the T790M allele in a high background of EGFR wild type. Using ddPCR, a researcher in the biotechnology industry was able to develop a highly accurate assay to quantify T790M concentration.
High sensitivity of the QX200 system allows quantification of **BRAF V600E** mutation in the presence of wild-type DNA using PrimePCR ddPCR assays. A, 2-D fluorescence amplitude plot generated by QuantaSoft software shows triplicate wells of a mixed mutant:wild-type sample. The black cluster on the plot represents the negative droplets, the green cluster represents the droplets that are positive for wild-type DNA only, the blue cluster represents the droplets that are positive for mutant DNA only, and the orange cluster represents the droplets that are positive for both mutant and wild-type DNA. B, 2-D fluorescence amplitude plot shows three replicates of a wild-type–only sample. C, fractional abundance plot shows the percentage frequency (orange markers) of the mutant DNA in a wild-type DNA background. The blue markers indicate the concentration of mutant DNA (copies/µl) and the green markers indicate the concentration of wild-type DNA (copies/µl) in each of three replicate samples. All error bars are generated by QuantaSoft software and represent the 95% confidence interval.
Precisely quantifying viral load is crucial for characterizing disease states and developing and validating therapies. Viral reservoirs fluctuate, sometimes dropping to very low levels that are nonetheless significant. Obtaining exact and reproducible measurements of viral DNA or RNA often represents the difference between success and failure in studying many different pathogens.

The QX200 system has the sensitivity to register extremely minute quantities of viral genetic material and distinguish it from complex mixtures. Investigators can obtain actual copy numbers of viral genome by generating complementary DNA through reverse transcription. Along with precision and reliability, the QX200 system also offers users the throughput needed to process samples with great efficiency.

**SUCCESS STORY**

A researcher compared quantitative PCR (qPCR) and ddPCR approaches for detecting residual human immunodeficiency virus (HIV) infection in a clinical sample set, which included an infant who was functionally cured of HIV. The researcher found that ddPCR was five times more sensitive than qPCR when measuring HIV DNA copies/million cells, and more than 20 times more accurate when measuring viral long terminal repeats (2-LTR circles).
ddPCR enables precise and reproducible detection of herpes simplex virus 1 (HSV-1) and β2 microglobulin (B2M) targets in a duplex assay. **A,** 2-D fluorescence amplitude plot shows three merged replicate samples of 10,000 copies/well of HSV-1 duplexed with 20,000 copies/well of B2M. The black cluster on the plot represents the negative droplets, the green cluster represents the droplets that are positive for HSV-1 only, the blue cluster represents the droplets that are positive for B2M only, and the orange cluster represents the droplets that are positive for both HSV-1 and B2M targets. **B,** concentration plot shows merged triplicate wells across an HSV-1 sample dilution series from 0 to 40,000 copies in a constant background of 20,000 copies (66 ng human genomic DNA) of B2M. The blue markers indicate HSV-1 copies/µl and the green markers indicate B2M copies/µl. All error bars are generated by QuantaSoft software and represent the 95% confidence interval.
WHAT'S IN A DROPLET?

DISTINGUISHING GENOMIC VARIATIONS

Copy number variation (CNV) is a prominent source of interindividual variability in the human genome. CNV has also been associated with cancers, neurological and autoimmune diseases, and adverse drug responses. Reliably identifying such CNVs represents an important capability in cutting-edge research.

The major technical hurdle in copy number (CN) assessment is the ability to discriminate, with statistical confidence, between consecutive CN states. Fundamentally, as CN state increases, the percentage difference in target genomic material between states decreases, making it harder to measure CNV at higher orders. By partitioning the amplification reaction across thousands of droplets, the QX200 system can resolve high consecutive copy number states (such as five vs. six) with an excellent degree of statistical confidence.

SUCCESS STORY

A researcher investigating a highly duplicated gene involved in human brain size evolution needed a method to validate small CN variations in test samples. Droplet Digital PCR proved much more effective than other methods the researcher had previously used, showing greater precision in detecting small differences at high CN states, as well as excellent reproducibility. These improvements in CNV quantification will allow the researcher to ask more complex questions regarding neurogenetic disorders such as micro/macrocephaly, schizophrenia, and autism.
The QX200 system provides superior resolution with copy number (CN) calls from CN1 to CN13. A. 2-D fluorescence amplitude plot shows duplicate wells of a CN6 sample duplexed with myelocytomatosis oncogene (MYC) and β-globin (HBB) assays. The black cluster on the plot represents the negative droplets, the green cluster represents the droplets that are positive for MYC only, the blue cluster represents the droplets that are positive for HBB only, and the orange cluster represents the droplets that are positive for both MYC and HBB targets. B, copy number plot shows samples ranging from CN1 to CN13 with precise duplicate well values. All error bars are generated by QuantaSoft software and represent the 95% confidence interval. NTC, no template control.
WHAT’S IN A DROPLET?

NGS VALIDATION AND LIBRARY QUANTIFICATION

Bio-Rad’s ddPCR platform can be easily incorporated into the next-generation sequencing (NGS) library preparation workflow to precisely quantify and balance sequencing libraries on sequencers. In addition to precise quantification, the data plots generated by the QX200 system are rich with qualitative library information, which is not available with other currently available methodologies. Digital PCR enables consistent library loading and efficient utilization of next-generation sequencers. After the NGS run, ddPCR can also be used to validate the results obtained by NGS, including genomic alterations such as single nucleotide polymorphisms, mutations, and copy number variations.

GENE expression studies

With its high sensitivity, the QX200 system is especially well-suited for discerning and quantifying rare transcripts, offering a better understanding of actual RNA function. With its high precision, the QX200 system can be used to detect small fold changes in expression of a target gene between samples, providing insights into the gene’s physiological implications. The ability to measure absolute values of RNA molecules adds a new level of insight to gene expression studies. The QX200 ddPCR system allows for such measurements without requiring a standard curve.

SUCCESS STORY

A research group used ddPCR to accurately quantify five NGS libraries of varying concentration. Bio-Rad’s ddPCR platform allowed the researchers to achieve an optimal cluster density of approximately 800,000/mm² required for sequencing on the Illumina MiSeq platform. Tests for library balancing showed that libraries could be balanced to within less than 15% of each other with a confidence interval of 95%.

SUCCESS STORY

A researcher used ddPCR to study the differential expression of huntingtin (HTT) mRNA encoded by both wild-type and mutant HTT genes. Quantifying these different transcripts of RNA gave the researcher more insight into their cellular functions, and laid the groundwork for better understanding disease mechanisms.
ddPCR provides nearly 1,000-fold greater sensitivity and an amplification bias–free approach compared to RNA-Seq for validation. A, concentration plot shows a twofold dilution of cDNA generated using Bio-Rad’s iScript™ advanced cDNA synthesis kit for RT-qPCR. Two independent measurement sets were made: one at a high concentration range and one at a lower range, with four points overlapping. B, table shows comparative results between ddPCR and MiSeq. ddPCR detected thousands of copies per well with low-abundance transcripts (TBP and GUSB genes) using an input of 100 ng total RNA, which demonstrates enhanced sensitivity. The MiSeq sequencer detected only single-digit reads per kilobase per million reads (RPKM) for these transcripts. ddPCR, Droplet Digital PCR.

For more information, visit www.bio-rad.com/QX200.
WHAT’S IN A DROPLET?

ABSOLUTE QUANTIFICATION WITH EVAGREEN

Through droplet partitioning and statistical analysis, Droplet Digital PCR provides absolute quantification without the need for a standard curve, bringing substantial improvements to many applications that require detection of low-abundance target copies or distinction of small fold changes between samples. Combining ddPCR with the power of EvaGreen detection chemistry enables genotyping and gene expression analysis for low-level target genes. The QX200 ddPCR system enables absolute quantification using EvaGreen chemistry, making such assays ideal for target DNA measurement, viral load analysis, and microbial quantification.

VALIDATED ddPCR ASSAYS

Bio-Rad’s PrimePCR ddPCR assays are fully wet-lab validated assays, expertly designed to provide single-copy PCR resolution without the use of a standard curve. These mutation detection and copy number assays have been validated specifically for digital PCR.

Bio-Rad’s PrimePCR real-time primer assays can also be used on the QX200 Droplet Digital PCR system with QX200 ddPCR EvaGreen supermix. Choose from more than 40,000 existing human and mouse gene expression assays.

SUCCESS STORIES

Precision cancer medicine involves rapid assessment of cancer mutations, with certain genetic errors serving as indicators for a specific therapy. A researcher was challenged with the composition and degradation of clinical samples that were used for assessment of FLT3 mutations for gastric tumors (sporadic diffuse). By partitioning the samples’ DNA templates, ddPCR removed the effects of PCR inhibitors and bias in the data, resulting in a more reliable quantification of the mutations present in the samples.

Designing an optimal probe assay may pose challenges and delay generation of reliable data. A researcher spent a great deal of time trying to design probes and primers for EGFR L858R, a receptor for epidermal growth factor, which is associated with lung tumors. Using the fully validated PrimePCR ddPCR mutation detection probe assay for EGFR L858R eliminated these tedious optimization steps, allowing the researcher to focus on the biology of the investigation, rather than assay design.
**ABSOLUTE QUANTIFICATION USING EVAGREEN**

**A**

ddPCR enables precise and reproducible detection calls across a range of sample concentrations. A concentration plot shows data for both merged wells (solid blue markers) and individual replicate wells (open blue markers) across a dilution series. Interleukin 1 (IL-1) samples ranging from 0 to 50,000 copies were detected with an EvaGreen assay and plotted on a log scale. There is excellent linearity and reproducibility between replicates.

**B**

1-D fluorescence amplitude plot shows a sample dilution curve detected with an EvaGreen IL-1 assay in the FAM/EvaGreen channel of the QX200 droplet reader. The data demonstrate good separation between positive (●) and negative (■) data points. All error bars are generated by QuantaSoft software and represent the 95% confidence interval. NTC, no template control.

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**DETECTION OF EGFR L858R MUTATION**

**A**

PrimePCR ddPCR assays are fully validated for the detection of EGFR L858R mutation. A 2-D fluorescence amplitude plot shows triplicate wells of a mixed mutant: wild-type sample. The black cluster on the plot represents the negative droplets, the green cluster represents the droplets that are positive for wild-type DNA only, and the orange cluster represents the droplets that are positive for mutant DNA only.

**B**

Fractional abundance plot shows the percentage frequency (orange markers) of the mutant DNA in a wild-type DNA background. The blue markers indicate the concentration of mutant DNA (copies/µl) and the green markers indicate the concentration of wild-type DNA (copies/µl) in each of three replicate samples. All error bars are generated by QuantaSoft software and represent the 95% confidence interval.
More Droplet Digital PCR Resources
Visit www.bio-rad.com/N/QX200 for a wide range of videos, technical reports, published findings, and other resources describing Bio-Rad’s unique Droplet Digital PCR technology and the QX200 ddPCR system.

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<tr>
<th>Specifications</th>
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<td>QX200 Droplet Generator</td>
<td>186-3021</td>
<td>One-Step RT-ddPCR Kit for Probes, 2 ml (2 x 1 ml), 200 x 20 µl reactions, 2x RT-ddPCR mix, includes 1 manganese acetate tube</td>
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Ordering Information

Catalog # | Description
---|---
QX200 Droplet Digital PCR System | 186-4001
QX200 Droplet Digital PCR System, includes droplet generator, droplet reader, laptop computer, software, associated component consumables | |
QX200 Droplet Generator | 186-4002
QX200 Droplet Generator, includes droplet generator, 1 pkg of 24 DG8 cartridges, 1 pkg of 24 DG8 gaskets, 2 cartridge holders, 1 power cord | |
QX200 Droplet Reader | 186-4003
QX200 Droplet Reader, includes droplet reader, ddPCR manual, 2 plate holders, USB cable, power cord | |
QX200 Drop Generator Cartridges and Gaskets | 186-4007
QX200 Drop Generator Cartridges and Gaskets, includes 5 pkg of 24 DG8 cartridges, 5 pkg of 24 DG8 gaskets | |
DG8 Cartridges for QX100/QX200 Droplet Generator | 186-4008
DG8 Cartridges for QX100/QX200 Droplet Generator, 1 pkg of 24 cartridges | |
DG8 Gaskets for QX100/QX200 Droplet Generator | 186-4009
DG8 Gaskets for QX100/QX200 Droplet Generator, 1 pkg of 24 gaskets | |
DG8 Cartridge Holder | 186-4010
DG8 Cartridge Holder | |
DG8 Reaction Oil for Probes | 186-4011
DG8 Reaction Oil for Probes, 10 x 7 ml | |
DG8 Reaction Oil for EvaGreen | 186-4012
DG8 Reaction Oil for EvaGreen, 2 x 7 ml | |
ddPCR Reagents | 186-4013
ddPCR Reagents | |
ddPCR Supermix for Probes (no dUTP) | 186-4014
ddPCR Supermix for Probes (no dUTP), 2 ml (2 x 1 ml), 200 x 20 µl reactions, 2x supermix | |
ddPCR Supermix for Probes | 186-4015
ddPCR Supermix for Probes, 25 ml (5 x 5 ml), 2,500 x 20 µl reactions, 2x supermix | |
ddPCR Supermix for Probes | 186-4016
ddPCR Supermix for Probes, 50 ml (10 x 5 ml), 5,000 x 20 µl reactions, 2x supermix | |
ddPCR Supermix for Probes | 186-4017
ddPCR Supermix for Probes (no dUTP), 2 ml (2 x 1 ml), 200 x 20 µl reactions, 2x supermix | |
ddPCR Supermix for Probes | 186-4018
ddPCR Supermix for Probes, 5 ml (5 x 1 ml), 500 x 20 µl reactions, 2x supermix | |
ddPCR Supermix for Probes | 186-4019
ddPCR Supermix for Probes (no dUTP), 5 ml (5 x 5 ml), 500 x 20 µl reactions, 2x supermix | |
ddPCR Supermix for Probes | 186-4020
ddPCR Supermix for Probes, 5 ml (5 x 1 ml), 2,500 x 20 µl reactions, 2x supermix | |
ddPCR Supermix for Probes | 186-4021
ddPCR Supermix for Probes (no dUTP), 5 ml (5 x 5 ml), 2,500 x 20 µl reactions, 2x supermix | |