QX200™ Droplet Generator

Instruction Manual

BIO-RAD

QX200 Droplet Generator

Instruction Manual

Catalog Number 1864002

Bio-Rad Technical Support Department

The Bio-Rad Technical Support department is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

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Revision History

Document	Date	Description of Change
QX200 Droplet Generator Instruction Manual DIR D094813 (10031907) Ver G	July 2023	Update the legal statement. Add the Managing Waste section. In the Order Information section, replace the word “laptop” with “computer," update products and catalog numbers, and remove reference to QX Manager. Update with other minor edits.

Table of Contents

Safety and Regulatory Compliance

This section cites regulatory requirements for laboratory and electrical equipment, as well as requirements for working with chemicals and hazardous substances, and also explains safety precautions and recommendations.

Important: Only trained personnel should use this instrument.

Regulatory Compliance

The instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2010 (3rd:2016, EN61010-1:2010 (3rd ed.). Electrical equipment for measurement, control, and laboratory use - Part 1: General requirements
- IEC 61326-1:2012 (Class A), EN 61326-1:2013 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements
- IEC 61010-2-081:2015, 3.0 edition, UL 61010-2-081:2015, CAN/CSA C22.2 No. 61010-2-081:19. Safety requirements for electrical equipment for measurement, control, and laboratory use. Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes (includes Amendment 1)
- CAN/CSA 22.2 No 61010-1-04, Safety requirements for electrical, equipment for measurement, control, and laboratory use, Part 1: General requirements
- CAN/CSA C22.2 No. 61010-2-101:2019 IEC 61010-2-101: 2018 (3rd ed.)
- Restriction of hazardous substances (ROHS) directive (European Union)
- Registration, evaluation, authorization and restriction of chemicals (REACH). European Chemicals Agency (ECHA) June 1, 2007
- Waste electrical and electronic equipment (WEEE) directive

This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.

Table 1. Regulatory symbols

Symbol	Definition
CE	The CE symbol indicates that the manufacturer ensures the product conforms with the essential requirements of the applicable EN directives.
C US	The CSA symbol indicates that a project has been tested to Canadian and U.S. standards, and it meets the requirements of those applicable standards. This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.
2012/19/EU www.bio-rad.com	The Waste Electrical and Electronic Equipment (WEEE) Directive symbol indicates that when the end-user wishes to discard this product, it must be sent to separate collection facilities for recovery and recycling.

Safety Warning Labels

Warning labels alert you about sources of injury or harm. Table 2 defines each safety warning label.

Table 2. Meaning of safety warning labels

Icon	Meaning
	**Warning about risk of harm to body or equipment** Operating the systerm before reading this manual can constitute a personal injury hazard. For safe use, do not operate this instrument in any manner unspecified in this manual. Only qualified laboratory personnel trained in the safe use of electrical equipment should operate this instrument. Always handle all components of the system with care and with clean, dry hands.
	**Warning about handling biohazardous materials** When handling biohazardous samples, adhere to the recommended precautions and guidelines and comply with any local guidelines specific to your laboratory and location.
	**Warning about risk of electric shock** In order to prevent electric shock, use caution when plugging and unplugging the instrument. Always turn off and unplug the instrument when performing maintenance procedures.

Safe Use Specifications

For safe operation of the instrument, Bio-Rad™ strongly recommends that you comply with instructions listed in this section.

This instrument is intended for laboratory use only. Bio-Rad is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or by modifications to the instrument not performed by Bio-Rad or an authorized agent.

- This instrument is for use only by trained personnel.
- Use only the power cord, power switch, and USB port supplied with the instrument, and the plug adapter corresponding to the electrical outlets in your region.
- Position the instrument on a solid, stable surface, with adequate room at the back and on each side so that users can easily reach the power cord and USB port.
- This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the provided instructional documentation, may cause harmful interference to radio communications. Operation of the systems in a residential area is likely to cause harmful interference, in which case users will be required to correct the interference at their own expense.

Note: Bio-Rad recommends maintaining a backup power source in case of power outages. A universal power supply (UPS) can protect from brown outs and power surges, while a regular backup generator does not.

Personal Protective Equipment

Proper use of gloves is recommended with use of oils and sample plates. OSHA requirements for PPE are set forth in the Code of Federal Regulations (CFR) at 29 CFR 1910.132 (General requirements); 29 CFR 1910.138 (Hand protection); 29 CFR 1926.95 (Criteria for standard personal protective equipment). Any gloves with impaired protective ability should be discarded and replaced. Consider the toxicity of the chemicals and factors such as duration of exposure, storage, and temperature when deciding to reuse chemically exposed gloves.

Listed below are features to aid glove selection for handling of machines, assays, oils, and cleaning solvents:

- Butyl gloves are made of a synthetic rubber and protect against peroxide, hydrofluoric acid, strong bases, alcohols, aldehydes, and ketones
- Natural (latex) rubber gloves are comfortable to wear and feature outstanding tensile strength, elasticity, and temperature resistance
- Neoprene gloves are made of synthetic rubber and offer good pliability, finger dexterity, high density, and tear resistance; they protect against alcohols, organic acids, and alkalis
- Nitrile gloves are made of copolymer and provide protection from chlorinated solvents such as trichloroethylene and tetrachloroethene; they offer protection when working with oils, greases, acids, and caustic substances

Hazards

The instrument is designed to operate safely when used in the manner prescribed by the manufacturer. If the instrument or any of its associated components is used in a manner not specified by the manufacturer, the inherent protection provided by the instrument may be impaired.

Bio-Rad Laboratories, Inc. is not liable for any injury or damage caused by the use of this equipment in any unspecified manner, or by modifications to the instrument not performed by Bio-Rad or an authorized agent. Only trained Bio-Rad personnel should perform service on the system.

Biohazards

The instrument is a laboratory product. However, if biohazardous samples are present, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location.

Note: No biohazardous substances are expended during normal operations of this instrument.

General Precautions

- Always wear laboratory coat, laboratory gloves, and safety glasses with side shields or goggles.
- Keep your hands away from your mouth, nose, and eyes.
- Completely protect any cut or abrasion before working with potentially infectious materials.
- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory.
- Store all infectious or potentially infectious material in unbreakable leak-proof containers.
- Before leaving the laboratory, remove protective clothing.
- Change gloves frequently. Do not use a gloved hand to write, answer the telephone, turn on a light switch, or touch anything that other people may touch without gloves. Remove gloves immediately when they are visibly contaminated.
- Do not expose materials that cannot be properly decontaminated to potentially infectious material.
- Upon completion of an operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant (for example, a 1:10 dilution of household bleach).

Disposal of Biohazardous Material

Dispose of the following potentially contaminated materials in accordance with laboratory local, regional, and national regulations:

- Clinical samples
- Reagents
- Used reaction vessels or other consumables that may be contaminated

Chemical Hazards

The instrument contains no potentially hazardous chemical materials.

Explosive or Flammability Hazards

The instrument poses no uncommon hazard related to flammability or explosion when used in a proper manner as specified by Bio-Rad.

Electrical Hazards

The instrument poses no uncommon electrical hazard to operators if installed and operated properly without physical modification and connected to a power source of proper specification.

Surface Decontamination

WARNING! To prevent electrical shock, always turn off and unplug the instrument before performing decontamination procedures.

Important: Do not use abrasive or corrosive detergents or strong alkaline solutions. These agents can scratch surfaces and damage the system.

The following areas can be cleaned with 10% bleach solution:

- Outer area and chassis
- Inner plate holders
- surfaces

To prepare and apply the disinfectant, refer to the instructions provided by the product manufacturer. For questions regarding the use of other cleaning agents, contact Bio-Rad Technical Support.

Important: Do not clean the handler Y-axis rail when the front door is open. This is a lubricated surface, and failures will occur if the lubrication is removed.

Decommissioning and Disposal

The instrument contains electrical materials that should be disposed of as unsorted waste and must be collected separately, according to European Union Directive 2012/19/EU on waste electrical and electronic equipment — WEEE Directive. The purpose of decommissioning is to make sure that the equipment is electrically and environmentally safe for disposal. Before disposal, contact your local Bio-Rad representative for country-specific instructions.

Transport

You must perform the specified decontamination procedures before moving or shipping the system. Always move or ship the instrument with the supplied packaging materials, which will protect the instrument from damage. If appropriate containers cannot be found, contact your local Bio-Rad office.

Warranty

Important: The instrument is for research use only, and not for use in diagnostic procedures.

The instrument and its associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad office for the details of the warranty. Follow the safety specifications listed in this chapter and throughout this guide.

The instrument is intended for laboratory use only. Bio-Rad Laboratories, Inc. is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent. Alteration of this instrument voids the warranty and safety certification, as it creates a potential safety hazard.

Use of unapproved supermixes or additives may harm the instrument and voids the warranty.

Use only the power cord supplied with the instrument, using only the plug adaptor that corresponds to the electrical outlets in your region.

Chapter 1 Introduction to Droplet Digital PCR

Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction method based on water-oil emulsion droplet technology. Droplet Digital PCR™ uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions.

ddPCR is highly effective in the following areas:

- **Absolute quantification** — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification.
- **Genomic alterations such as gene copy number variation (CNV)** — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number.
- **Detection of rare sequences** — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences.
- **Gene expression and microRNA analysis** — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision.
- **Next-generation sequencing (NGS)** — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification.
- **Single cell analysis** — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells.
- **Genome edit detection** — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

ddPCR has the following benefits for nucleic acid quantification:

- **Unparalleled precision** — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured.
- **Increased signal-to-noise ratio** — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a ±10% precision in quantification.
- **Removal of PCR efficiency bias** — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero.
- **Simplified quantification** — There is no requirement for a standard curve for absolute quantification.

ddPCR Workflow

The ddPCR process conforms to the following workflow, and the full process is performed on one well before moving to the next:

- You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix.
- A droplet generator fractionates a sample into droplets with target and background DNA distributed randomly into the droplets during the partitioning process.
- Following droplet generation, the droplets are run through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet.
- A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample and uses Poisson statistical formulas to determine the absolute quantity.

  Note: Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets.

Finding Out More

Go to https://bio-rad.com to access links to technical notes, manuals, videos, product information, and technical support. The website also provides many technical resources on a wide variety of methods and applications related to PCR, Droplet Digital PCR, and gene expression.

Chapter 2 About the QX200 Droplet Generator

The QX200™ Droplet Generator uses microfluidics to combine oil and sample to create the droplets required for ddPCR analysis. The instrument generates droplets from up to eight samples in a DG8 cartridge at a time in about 2 min. You can generate droplets in one cartridge at a time.

Following reaction preparation using the appropriate ddPCR supermix, the prepared samples and droplet generation oil (or buffer for wells without sample) are transferred to the DG8 cartridge. The loaded cartridge is covered with a gasket and placed in the QX200 Droplet Generator, which combines the samples and oil within the microchannels of the cartridge to create an emulsion of approximately 20,000 monodisperse, nanoliter-sized droplets for each sample.

Included Items

This section identifies the functional items included and shipped with your purchase.

Table 3. QX200 Droplet Generator included items

Component	Description	Catalog Number
QX200 Droplet Generator	Instrument used for droplet generation	1864002
Power cord	U.S. standard power cord with grounded plug (type 5-1SP) and CS connector (10A/125V), and power supply to 5mm DC power jack inlet	Contact Bio-Rad Technical Support
DG8 droplet generator cartridges and gaskets	Microfluidic cartridge used to mix sample and oil to generate droplets; gaskets seal the cartridge to prevent evaporation and apply pressure required for droplet formation	1864007 (pkg of 24)
Droplet generator cartridge holder	Positions and holds the droplet generator cartridge in the instrument for droplet generation	1863051

Instrument Specifications

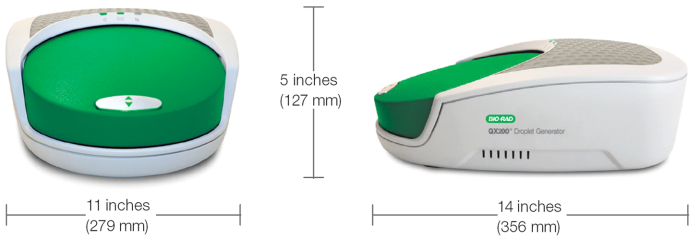


Table 4. Instrument size and weight

Specification	Description
Weight	10 lb (4.5 kg)
Size (W x D x H)	11 in x 14 in x 5 in (28 cm x 36 cm x 13 cm)

For information on required environmental conditions, see Environmental Requirements on page 22.

Additional Components

The following tables identify additional components you can purchase for use with your system or instrument.

Table 5. Accessories and consumables

Component	Description	Catalog Number
ddPCR 96-Well Plates	Clear well/clear shell semi-skirted plates	12001925 (pkg of 25)
DG8 Cartridge Holders	Droplet generation cartridge holders for the QX200 Droplet Generator	1863051
DG8 Cartridges and Gaskets	Droplet generation cartridges and gaskets for the QX200 Droplet Generator	1864007 (pkg of 24)
DG8 Cartridges	Droplet generation cartridges for the QX200 Droplet Generator	17005222 (pkg of 24)
DG8 Gaskets	Gaskets for the DG8 cartridges	17005223 (pkg of 24)
Droplet Generation Oils	■ Droplet Generation Oil for Probes, 70 ml ■ Droplet Generation Oil for EvaGreen®, 14 ml ■ Droplet Generation Oil for EvaGreen®, 70 ml Droplet generation oils for the QX200 Droplet Generator.	1863005 (10 x 7 ml) 1864005 (2 x 7 ml) 1864006 (10 x 7 ml)
Droplet Reader Oil	ddPCR droplet reader oil for QX600 and QX200 instruments and systems	1863004
Rainin Pipets	■ 20 µl pipet for sample loading ■ 50 µl pipet for droplet transfer ■ 8-channel 200 µl pipet for oil	Rainin L-20 Rainin L-50, L8-50 Rainin L8-200
Rainin Pipet Tips	Filtered	Rainin GP-L10F Rainin GP-L200F
Reagent trough	Any	N/A
8-cap strips	Any	N/A

Table 6. Thermal cyclers and plate sealer

Product	Description	Catalog Number
C1000 Touch Thermal Cycler with 96-Deepwell Reaction Module	Includes the C1000 Touch Thermal Cycler chassis, 96-deepwell reaction module, USB flash drive	1851197
PTC Tempo Deepwell Thermal Cycler	Includes the thermal cycler, power cord, USB cable, and Ethernet cable	12015392
PX1 PCR Plate Sealer	PCR plate sealer, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord	1814000
Pierceable Foil Heat Seal	Heat seal for the PX1 PCR Plate Sealer	1814040

Table 7. ddPCR supermixes

Supermix	Description	Catalog number	
ddPCR EvaGreen® Supermix	2x supermix, for use in nucleic acid sample preparation with the QX600 and QX200 ddPCR systems	1864033 1864034 1864035 1864036	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1ml) 50ml (50 x 1ml)
ddPCR Supermix for Probes (no dUTP)	2x supermix, for use in nucleic acid sample preparation with the QX600™ and QX200 ddPCR systems	1863023 1863024 1863025	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1)
ddPCR Supermix for Probes	2x supermix, for use in sample preparation with the QX600 and QX200 ddPCR systems	1863026 1863010 1863027 1863028	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1ml) 50ml (50 x 1ml)
ddPCR Multiplex Supermix	4x supermix especially suited for probe-based detection of multiple targets in DNA samples using the QX600 and QX200 ddPCR systems	12005909 12005910 12005911	1.2ml (2 x 0.6ml) 3 ml (5 x 0.6 ml) 12.5 ml (5 x 2.5 ml),

Table 7. ddPCR supermixes, continued

Supermix	Description	Catalog number	
ddPCR Supermix for Residual DNA Quantification	2x supermix, for use in residual DNA detection with the QX600 and QX200 ddPCR systems	1864037 1864038 1864039 1864040	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1ml) 50ml (50 x 1ml)
One-step RT-ddPCR Advanced Kit for Probes	200 or 500x 20 µl reaction kit, for absolute quantification of target RNA in a one-step format with the QX600 and QX200 ddPCR systems	1864021 1864022	2ml (2 x 1ml) 5ml (5 x 1ml)

Table 8. Buffer controls

Buffer	Catalog Number
ddPCR Buffer Control Kit for Probes	1863052
QX200 Buffer Control Kit for EvaGreen®	1864052

Environmental Requirements

Table 9 lists the environmental requirements for the QX200 Droplet Generator.

Important: You must use the supplied shielded cables with your instrument to ensure compliance with the Class A FCC limits.

Table 9. Conditions for safe use

Usage aspect	Requirements
Rated input power	100–240 V, 50–60 Hz, (plugs into standard AC receptacle)
Voltage fluctuations	± 10% for the included external power supply **Note:** Use only the power cord supplied with the equipment.
Pollution degree	2
Usage temperature	18–30° C
Relative humidity	non-condensing
Altitude	0 to above sea level
Installation category	II (external power supply plugs into a standard AC receptacle) Indoor use only
Ventilation requirements	The following distances should be unobstructed for proper ventilation:

General Maintenance

When surfaces of the instrument require general cleaning, use deionized/distilled water to wipe down with a slightly dampened cloth. For decontamination, you can use 10% bleach followed by 70% ethanol and/or deionized/ distilled water.

Important: Do not use acetone or tap water.

Inspect the equipment regularly for damaged external components or wiring. Do not use if damaged.

Note the following:

- Bio-Rad droplet generation and reader fluids are based on fluorinated hydrocarbon chemistry, and should be disposed of in accordance with institutional, state and local regulations.
- Avoid release to the environment and prevent entry into sewer systems or bodies of water.
- As a disposal alternative, incinerate in a permitted high-temperature waste incineration facility.
- Do not use the droplet generator with biohazardous material.
- Do not replace detachable power cord with an uncertified or an inadequately rated cord.

Managing Waste

You must apply standard Material Safety Data Sheet (MSDS) and OSHA practices when handling and disposing of generated waste. A typical waste profile should contain the following:

Table 10. Managing Waste

Chemical name	Weight %
Fluorinated fluids	>50%
Water	<50%
Bleach	<10%
Other materials (proteins, nucleic acids, fluorescent dye)	<10%

Note: The chemical identities and exact percentages in the above table are withheld as trade secrets.

Bio-Rad droplet generation and reader fluids are based on fluorinated hydrocarbon chemistry, and should be disposed of in accordance with institutional, state and local regulations. Avoid release to the environment and prevent entry into sewer systems or bodies of water. As a disposal alternative, incinerate in a permitted high-temperature waste incineration facility.

These nonflammable fluids are inert and have low environmental impact and low toxicity.

Toxicological Information

Chemical name: (mixture)
Oral LD50: >5000 mg/kg (Rat)
Dermal LC50: >5000 mg/kg (Rabbit)
Inhalation LD50: >8838 ppm (Rat/8-hr)

Ecological Information

Acute aquatic hazard: LC50 > 500 mg/l (96-hr./static; Pimephales promelas/Fathead minnow)
Chronic aquatic hazard: No information available (GHS Chronic 4)

Connecting the QX200 Droplet Generator

To connect the instrument

1. Position the instrument so it can be easily disconnected from the power source.
2. For proper ventilation, leave 10 in (25.4 cm) clear space behind the instrument, and 5 in (12.7 cm) clear space to the right and left.
3. Ensure the ground is reliably connected before plugging in the instrument.
4. Connect the instrument to the power source to power it on.

   The status indicator turns solid green to indicate power is on.

   Important: Use only the power cord and power adapter provided.
5. To open and close the lid, press the button on the lid.

Status Indicator Lights

This section describes the indicator lights on the top of the QX200 Droplet Generator.

Table 11. Droplet Generator Status Indicator Lights

Indicator	 (Power)	 (Cartridge)	 (Status)
Solid Green 	Power is on	Cartridge is positioned properly	Droplets generated; run complete
Flashing Green 	N/A	N/A	Droplets generating; run in progress
Flashing Amber 	N/A	N/A	Error during run (no seal, no gasket, or an empty well)
No color 	Power is off	No cartridge loaded	Instrument is idle

Chapter 3 Droplet Generation

Preparing the Sample

Prepare the PCR reaction by combining 2x PCR supermix, 20x primers and probe, and DNA sample. Mix by vortexing in short pulses, and then briefly centrifuge.

Note the following:

- The concentration of intact human genomic DNA should be less than 66 mg per 20 µl reaction. If using higher concentrations, digest DNA with a restriction endonuclease that does not cut target or reference amplicons.
- Use one of the PCR supermixes recommended in Appendix B, Ordering Information. These supermixes contain reagents required for droplet generation. Follow instructions in the product inserts to prepare the samples for droplet generation.
- Vortex the supermixes thoroughly to ensure homogeneity, since a concentration gradient may form during -20° C storage. Alternatively, pipet up and down more than 5 times to mix. Before dispensing, centrifuge briefly to collect contents at the bottom of the tube.
- Thaw and equilibrate reaction components to room temperature. If the sample is prone to thermal degradation, prepare the reaction mix on ice, but equilibrate the reaction mix to room temperature (approximately 3 min) before loading in the DG8 cartridge for droplet formation.
- Assemble reaction mixtures in vials or in 96-well PCR plates. Note that using a PCR plate, you can load samples into the DG8 cartridge using an 8-channel pipet.
- Use standard lab precautions to avoid contamination of the reaction mix and sample. Wear gloves, work in a clean area (such as a PCR hood) and use clean pipets and low protein binding tubes.

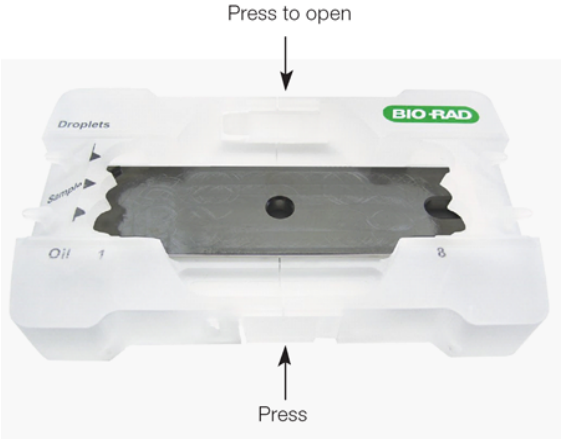
Operating the QX200 Droplet Generator

The instrument prepares droplets for up to eight samples at a time. Each DG8 cartridge contains eight sample wells. Each well must contain sample or 1x buffer control. Wells containing sample must also contain droplet generation oil. Droplet generation takes approximately 2 min for each full cartridge.

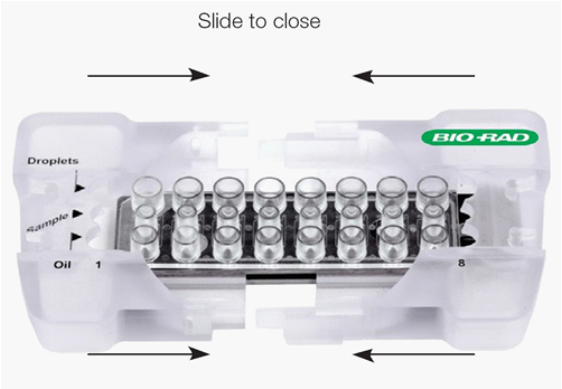
Inserting the Cartridge into the Cartridge Holder

To insert the cartridge

1. Press the latch on each side of the cartridge to open it.



2. Slide the cartridge to the right, and then drop it into the holder.
3. Press the halves of the holder together to close it.



Filling and Preparing the Cartridge for the Run

Air bubbles can cover the bottom of the well, resulting in 2,500 to 7,000 fewer droplets and poor data quality. Use the pipeting technique described in this section to

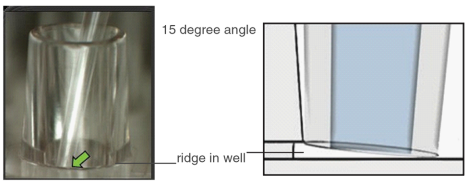
- Avoid air bubbles
- Ensure samples wet the bottom of the wells so they are wicked into microchannels (necessary for proper droplet generation)

To fill the cartridge

1. Transfer 20 µl of each prepared sample to the sample wells (middle row) of the DG8 cartridge.

   Important: Use only 20 µl aerosol-barrier (filtered) Rainin pipet tips. Do not use 200 µl pipet tips.

   a. Gently slide the pipet tip down the side of the wall at a 15-degree angle until it passes over the ridge near the bottom. .



   b. Holding the 15-degree angle, ground the pipet tip against the bottom edge of the sample well, while slowly dispensing a small portion of the sample.

      Do not pipet directly onto the side wall of the well.

   c. After dispensing about half the sample, slow draw the tip up the side wall while dispensing the rest of the sample.

      Do not push the pipet plunger past the first stop.

2. Dispense the droplet generation oil into the reagent trough.

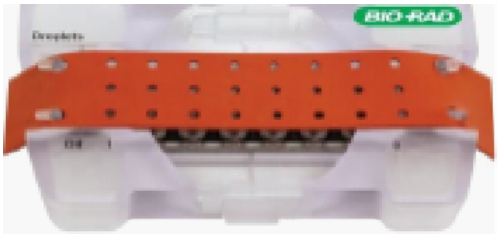
Use the following volume guide:

- For 8 wells, use 700 µl of oil
- For 24 wells, use 1,820 µl of oil
- For 48 wells, use 3,500 µl of oil
- For 96 wells, use 6,860 µl of oil

3. Using a multichannel pipet, fill each oil well (bottom row) with 70 µl of droplet generator oil from the trough.

To prepare the cartridge for the droplet generation run

▶ Hook the gasket over the cartridge holder using the holes on the sides.



Important: The gasket must be hooked securely on each end or the instrument cannot reach the required pressure.

Inserting the Cartridge and Generating Droplets

To insert the cartridge into the instrument and generate droplets

1. Press the button on the top of the QX200 Droplet Generator to open the instrument.

2. Place the cartridge (in the holder) into the instrument.

   When its position is correct, the power and cartridge status indicator lights turn green. For information on indicator lights, see Status Indicator Lights on page 25.

3. Press the button on the instrument again to close the lid.

   When the lid is closed, droplet generation begins. The droplet light on the right flashes green to indicate that droplet generation has begun.

   When droplet generation is complete, all lights are solid green.

4. Open the instrument and remove the cartridge holder and cartridge.
5. Remove and discard the disposable gasket.

   The top level of each well contains the droplets.

Appendix A Preparing For and Executing PCR

After you have generated droplets in the cartridge, you can pipet 40 µl of the contents of the top wells containing the droplets into a single column of a 96-well PCR plate. To avoid shearing or coalescing the droplets, use the following pipeting techniques Repeat until all droplets are transferred or the plate is full.

Aspirating Droplets from the Cartridge

To aspirate droplets from the droplet generation cartridge

1. Use an 8-channel manual L-50 pipet with 200 µl tips.

   Do not use wide or narrow-bore tips.

2. Place the cartridge holder on a flat surface and position the pipet tips in each of the 8 top wells at a ~30-45° angle to the junction where the side wall meets the bottom of the well.

   Do not position the pipet tip in a vertical orientation (90°) or against any flat surface of the well. Do not allow the tips to be flat against the well bottoms.

3. Slowly draw 40 µl of droplets into the pipet tip.

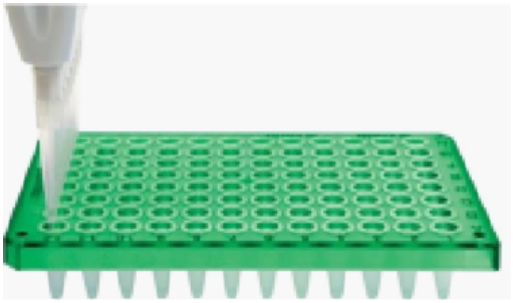
   This should take 5 sec, and ~5 µl air is expected. Do not aspirate more than 40 µl, as this causes air to percolate through the droplets.

4. Pipet slowly. Apply a stable resistive force to the plunger to draw and aspirate droplets smoothly into and out of pipet tips.

Dispensing Droplets into the Plate

To dispense droplets into the 96-well plate

1. Position the pipet tip along the side of the well, near—but not at—the bottom of the well, and then slowly dispense the droplets (5 sec).



2. To prevent evaporation and contamination with particulates, cover the plate (for example, with 8-cap strips or the lid from a pipet tip box) as you work.

Sealing the Plate

To seal the PCR plate with foil immediately after transferring droplets

Note: Use pierceable foil plate seals that are compatible with the PX1 PCR Plate Sealer and the needles in the QX200 Droplet Reader. For information, see PX1 PCR Plate Sealer Instruction Manual (Item #10023997).

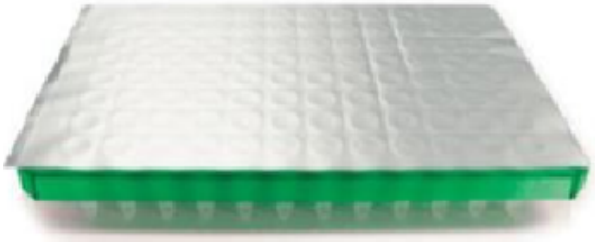
1. Set the plate sealer temperature to 180° C and the time to 5 sec.
2. Touch the arrow to open the PX1 tray door, and then position the support block on the tray with the 96-well side facing up.

3. Place the 96-well plate onto the support and ensure that all plate wells are aligned with the support block.
4. Cover the 96-well plate with one sheet of pierceable foil seal.

   Note: The yellow label on the Bio-Rad heat seal bag identifies the sealing surface.

   Important: Do not attempt to place the frame over the foil-covered plate. The frame is only for use with other seals.
5. Once the 96-well plate is secured on the support block and covered with the pierceable foil seal, touch the Seal button.

   The tray closes and heat sealing begins. When heat sealing is complete, the PX1 door opens automatically.
6. Remove the plate from the block, and then remove the block from the PX1.
7. Check that all wells in the plate are sealed.



   The depressions of the wells should be visible on the foil.

Important: Begin thermal cycling (PCR) within 30 min of sealing the plate, or store the plate at 4° C for up to 4 hr before thermal cycling. Refer to the supermix product for cycling conditions.

Thermal Cycling and Droplet Reading

To perform thermal cycling

1. Once the 96-well plate containing the droplets is sealed, place it into the thermal cycler for PCR amplification.

   Refer to the supermix product inserts for cycling conditions.

2. When PCR amplification is complete, remove the 96-well plate from the thermal cycler.

To perform droplet reading

1. Read the droplets using the QX200 or QX600™ Droplet Reader.

   For information, see the QX200 Droplet Reader and QX Manager Software, Standard Edition, User Guide (#1000107223) or the QX600 Droplet Reader and QX Manager Software, Standard Edition, User Guide (#10000153877).

2. If the goal is to read or quantify droplets and recover material from droplets in parallel, prepare two sets of reactions, one for each application.

   For example, in a single DG8 cartridge. four wells will be read after thermal cycling and four will not be read.

Appendix B Ordering Information

This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad ddPCR products, including your QX200 Droplet Generator.

ddPCR System and Instrument Packages

Table 12. ddPCR system and instrument packages

Product	Description	Catalog Number
QX200 Droplet Generator	Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1)	1864002
QX200 Droplet Reader	Includes the droplet reader, two plate holders, USB cable, and power cord	1864003
QX600 Droplet Reader	Includes the droplet reader, two plate holders, USB cable, and power cord	12013328

Thermal Cyclers and Plate Sealer

Table 13. Thermal cyclers and plate sealer

Product	Description	Catalog Number
C1000 Touch Thermal Cycler with 96-Deepwell Reaction Module	Includes the C1000 Touch Thermal Cycler chassis, 96-deepwell reaction module, USB flash drive	1851197
PTC Tempo Deepwell Thermal Cycler	Includes the thermal cycler, power cord, USB cable, and Ethernet cable	12015392
PX1 PCR Plate Sealer	PCR plate sealer, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord	1814000
Pierceable Foil Heat Seal	Heat seal for the PX1 PCR Plate Sealer	1814040

QX200 Droplet Generator Accessories

Product	Description	Catalog Number
DG8 droplet generator cartridges and gaskets	Microfluidic cartridges used to mix sample and oil for droplet generation, and gaskets to seal the cartridges **Note:** You must seal the cartridge to prevent evaporation and to apply required pressure for droplet generation.	1864007
Droplet generator cartridge holder	Positions and holds the droplet generator cartridge in the instrument compartment	1863051
Power cord	Connects the QX200 Droplet Generator to the power source **Note:** Contact Bio-Rad Technical Support for replacement information.	N/A

QX600 Droplet Reader Accessories

Table 14. QX600 Droplet Reader accessories

Product	Description	Catalog Number
Computer	Computer that connects to the QX600 Droplet Reader for data collection and analysis	12017458
USB cable and power cord	Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information.	Included
Droplet reader plate holders (2)	Used to position the 96-well plate in the droplet reader plate compartment	12006834

QX200 Droplet Reader Accessories

Table 15. QX200 Droplet Reader accessories

Product	Description	Catalog Number
Computer	Computer that connects to the QX200 Droplet Reader for data collection and analysis	12017458
USB cable and power cord	Cable connecting the computer to the instrument and power cord connecting the instrument to the power source **Note:** Contact Bio-Rad Technical Support for replacement information.	Included
Droplet reader plate holders (2)	Used to position the 96-well plate in the droplet reader plate compartment	12006834

ddPCR Consumables and Other Materials

Table 16. Consumables and other materials

Product	Description	Catalog Number
ddPCR 96-Well Plates	Cear well/clear shell semi-skirted plates	12001925 (pkg of 25)
Droplet Generation Oils	Droplet generation oil for the QX200 Droplet Generator:	
	■ Droplet Generation Oil for Probes, 10 x 7 ml	1863005
	■ Droplet Generation Oil for EvaGreen®, 2 x 7 ml	1864005
	■ Droplet Generation Oil for EvaGreen®, 10 x 7 ml	1864006
Rainin Pipets	■ 20 µl for sample loading	L-20, L8-20
	■ 50 µl for droplet transfer	L-50, L8-50
	■ 8-channel, 200 µl for oil	L8-200
Rainin Pipet Tips	Filtered	GP-L10F GP-L200F
Foil Plate Seals for PX1	Pierceable foil plate seals for PX1 Plate Sealer	1814040
ddPCR Droplet Reader Oil	Droplet reader oil for the QX600 Droplet Reader and QX200 Droplet Reader	1863004
Droplet reader waste bottle	You can use an empty droplet reader oil bottle to collect the waste from droplet reading.	N/A

ddPCR Supermixes

Table 17. ddPCR supermixes

Supermix	Description	Catalog number	
ddPCR EvaGreen® Supermix	2x supermix, for use in nucleic acid sample preparation with the QX600 and QX200 ddPCR systems	1864033 1864034 1864035 1864036	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1ml) 50ml (50 x 1ml)
ddPCR Supermix for Probes (no dUTP)	2x supermix, for use in nucleic acid sample preparation with the QX600™ and QX200 ddPCR systems	1863023 1863024 1863025	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1)

Table 17. ddPCR supermixes, continued

Supermix	Description	Catalog number	
ddPCR Supermix for Probes	2x supermix, for use in sample preparation with the QX600 and QX200 ddPCR systems	1863026 1863010 1863027 1863028	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1ml) 50ml (50 x 1ml)
ddPCR Multiplex Supermix	4x supermix especially suited for probe-based detection of multiple targets in DNA samples using the QX600 and QX200 ddPCR systems	12005909 12005910 12005911	1.2ml (2 x 0.6ml) 3 ml (5 x 0.6 ml) 12.5 ml (5 x 2.5 ml),
ddPCR Supermix for Residual DNA Quantification	2x supermix, for use in residual DNA detection with the QX600 and QX200 ddPCR systems	1864037 1864038 1864039 1864040	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1ml) 50ml (50 x 1ml)
One-step RT-ddPCR Advanced Kit for Probes	200 or 500x 20 µl reaction kit, for absolute quantification of target RNA in a one-step format with the QX600 and QX200 ddPCR systems	1864021 1864022	2ml (2 x 1ml) 5ml (5 x 1ml)

Buffer Controls

Table 18. Buffer controls

Buffer	Catalog Number
ddPCR Buffer Control Kit for Probes	1863052
QX200 Buffer Control Kit for EvaGreen®	1864052

ddPCR Kits

Table 19. ddPCR Kits

Product	Description	Catalog number
ddPCR Mutation Screening Kits		
ddPCR KRAS G12/G13 Screening Kit		1863506
ddPCR KRAS Q61 Screening Kit		12001626
ddPCR BRAF V600 Screening Kit		12001037
ddPCR NRAS Q61 Screening Kit		12001006
ddPCR NRAS G12 Screening Kit		12001094
ddPCR NRAS G12/G13 Screening Kit		12001627
ddPCR EGFR Exon 19 Deletions Screening Kit		12002392
	All kits include 20x multiplex assay and 2x ddPCR supermix for probes (no dUTP).	
ddPCR Residual DNA Quantification Kits		
ddPCR CHO Residual DNA Quantification Kit		17000031
ddPCR E.coli Residual DNA Quantification Kit		17000032
	200x 20 µl reactions, includes 20x CHO or E.coli RDQ assay and 2x ddPCR Supermix for Residual DNA Quantification.	
ddPCR Copy Number Determination Kits		
ddPCR SMN1 Copy Number Determination Kit		1863500
ddPCR SMN2 Copy Number Determination Kit		1863503
	200x 20 µl reactions, includes assay at 20x concentration, 2x ddPCR Supermix for Probes (no dUTP) and positive controls	
ddPCR Library Quantification Kit		
ddPCR Library Quantification Kit for Illumina TruSeq		1863040
	200x 20 µl reactions, includes 1 vial of primers and probes at 20x concentration, 2x ddPCR Supermix for Probes (no dUTP) and positive controls	

ddPCR Assays

Table 20. ddPCR Assays

Product	Catalog number	
ddPCR HDR Gene Edit Assay	12002312	100 rxns
ddPCR HDR Gene Edit Assay	12002313	500 rxns
ddPCR HDR Gene Edit Package	12003796	1,000 rxns
ddPCR HDR Ref Assay, Predesigned	12003805	100 rxns
ddPCR HDR Ref Assay, Predesigned	12003806	500 rxns
ddPCR HDR Ref Package, Predesigned	12003793	1,000 rxns
ddPCR NHEJ Gene Edit Assay	12002314	100 rxns
ddPCR NHEJ Gene Edit Assay	12002315	500 rxns
ddPCR NHEJ Gene Edit Package	12003794	1,000 rxns

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Life Science Group

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