QX200™ Droplet Generator

Instruction Manual

Catalog #186-4002

BIO-RAD

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Practice of the polymerase chain reaction (PCR) may require a license.

Safety and Regulatory Compliance

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

- IEC 61010-1:2001 (2nd ed.), EN61010-1:2001 (2nd ed). Electrical Equipment for Measurement, Control, and Laboratory Use — Part 1: General requirements
- EN 61326-1:2006 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements

This equipment generates, uses, and can radiate radiofrequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense.

The CE mark indicates that the manufacturer ensures the product conforms with the essential requirements of the applicable EC directives.

The CSA mark indicates that a product, process, or service has been tested to a Canadian or U.S. standard and it meets the requirements of the applicable CSA standard.

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.

The Waste Electrical and Electronic Equipment 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.

Instrument Safety Warnings

Alteration of this instrument voids the warranty and safety certification and creates a potential safety hazard. This instrument is intended for laboratory use only. Bio-Rad Laboratories 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. Follow the safety specifications listed here and throughout this manual. Use only the power cord supplied with the instrument, using only the plug adaptor that corresponds to the electrical outlets in your region. Use of unapproved supermixes may harm the instrument and voids the warranty.

PPE (Personal Protective Equipment) Training

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. 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

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1 QX200™ Droplet Generator

1.1 Introduction

The QX200™ Droplet Digital™ PCR (ddPCR™) system performs accurate and precise digital PCR. The system consists of two instruments, the QX200 droplet generator and the QX200 droplet reader, and their associated consumables. The QX200 droplet generator partitions samples into 20,000 nanoliter-sized droplets and, after PCR on a thermal cycler, droplets from each sample are analyzed individually on the QX200 droplet reader. PCR-positive and PCR-negative droplets are counted to provide absolute quantification of target DNA in digital form. Alternatively, amplified products can be extracted from droplets following PCR for downstream applications, such as sequencing or cloning.

The ddPCR system lets you:

- Detect rare DNA target copies with unmatched sensitivity
- Determine copy number variation with unrivaled accuracy
- Measure gene expression levels with precision

Applications and uses include:

- Copy number variation
- Rare sequence detection
- Gene expression analysis
- Next-generation sequencing (NGS) library quantification
- Viral load determination
- Single cell gene expression analysis
- Absolute quantification
- Rare mutant detection
- miRNA analysis
- NGS sample preparation
- GMO detection

This manual covers use of the QX200 droplet generator and preparation for PCR. For information on the QX200 droplet reader, please refer to bulletin 10031906.

1.2 QX200 Droplet Generator

The QX200 droplet generator uses microfluidics to combine oil and water (sample) to create the droplets required for ddPCR analysis. It generates droplets from up to eight samples at a time in about 2 minutes.

Following reaction preparation using the appropriate ddPCR supermix, 20 µl each of up to eight prepared samples (or blanks) and droplet generator oil are transferred to the droplet generator (DG8™) cartridge. The loaded cartridge is covered with a gasket and placed in the QX200 droplet generator. There, the samples and oil are combined within the microchannels of the cartridge to create an emulsion of ~20,000 monodisperse, nanoliter-sized droplets for each of the samples. Following droplet generation, the droplets are transferred to a standard 96-well PCR plate and amplified to end point using a standard thermal cycler.

When cycling is complete, the plate is loaded into the QX200 droplet reader. The droplet reader sips each sample, singulates the droplets, and streams them in single file past a two-color detector. The detector reads each droplet and determines which contain a target (+) and which do not (–). If quantitation of droplets is not required, PCR products can be extracted from droplets following thermal cycling for downstream applications, such as sequencing or cloning.

The QX200 droplet generator includes the components listed in Table 1.1. Additional requirements for droplet generation and PCR are listed in Table 1.2. For complete system requirements, refer to the QX200 Droplet Reader Instruction Manual (bulletin 10031906).

Table 1.1. QX200 droplet generator components. Catalog # refers to replacement items (quantities may be different).

Component	Description	Catalog #
QX200 droplet generator	Instrument used for droplet generation	186-4002
DG8 droplet generator cartridges and gaskets (24)	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	186-4007
Droplet generator cartridge holder	Positions and holds the droplet generator cartridge in the instrument for droplet generation	186-3051
Power cord	Connects QX200 droplet generator to power source	Call technical support

QX200 droplet generator.

Table 1.2. Additional materials required for droplet generation.

Component	Recommended	Catalog #
Reagents for probe detection		
PCR supermix	ddPCR supermix for probes	186-3010, 186-3026, 186-3027, 186-3028
	Droplet PCR supermix	186-3023, 186-3024, 186-3025
	One-Step RT-ddPCR supermix for probes	186-3021, 186-3022
Droplet generator oil	Droplet generator oil for probes	186-3030, 186-3005
Control	ddPCR buffer control kit for probes	186-3052
Reagents for EvaGreen detection		
PCR supermix	QX200™ ddPCR™ EvaGreen® supermix	186-4033, 186-4034, 186-4035, 186-4036
Droplet generator oil	QX200 droplet generator oil for EvaGreen dye	186-4005, 186-4006
Control	QX200 buffer control kit for EvaGreen dye	186-4052
Consumables and other materials		
Pipets	20 µl pipet for sample loading	Rainin L-20
	50 µl pipet for droplet transfer	Rainin L-50, L8-50
	8-channel, 200 µl pipet for oil	Rainin L8-200
Pipet tips	Filtered	Rainin GP-L10F, GP-L200F
96-well PCR plates	twin.tec semi-skirted 96-well plate	Eppendorf 951020362
Reagent trough	Any	
Foil plate seals	Pierceable foil plate seals	181-4040
Plate sealer	PX1™ PCR plate sealer	181-4000
8-cap strips	Any	

1.3 Installation and General Operation

- Connect the QX200 droplet generator to a power source using only the power cord provided. Ensure the ground is reliably connected before plugging in the instrument
- Leave 10" (5 cm) clear space behind and 5" (2.5 cm) clear to the right and left for proper ventilation
- Power on the droplet generator by plugging it in. The status indicator turns solid green to indicate power is on
- Open and close the instrument by pressing the button on top of the green lid

U.S. standard power cord set with grounded plug (Type 5-15P) and C5 connector (10 A/125 V)

Power supply to 5 mm DC power jack inlet

2 Droplet Generation

2.1 Sample Preparation

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

- The concentration of intact human genomic DNA should be <66 ng 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 Table 1.2, as these 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, as a concentration gradient may form during –20°C storage. Alternatively, pipet up and down >5 times to mix. Centrifuge briefly to collect contents at the bottom of the tube before dispensing
- 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 (~3 min) before loading into the DG8™ cartridge for droplet formation
- Assemble reaction mixtures in vials or in 96-well PCR plates. The advantage of using a PCR plate is that samples can be loaded 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

2.2 Operation of the QX200™ Droplet Generator

The QX200 droplet generator prepares droplets for up to eight samples at a time. Droplet generation takes ~2 minutes for each set of eight samples (~30 minutes for a 96-well plate).

- All 8 sample wells in the DG8 droplet generator cartridge must contain sample (or 1x buffer control), and all 8 oil wells must contain droplet generator oil
- Do not load sample or oil into the DG8 cartridge unless it is inserted in the holder

1. Insert the DG8 cartridge into the holder with the notch in the cartridge at the upper left of the holder:
   a. Open the cartridge holder by pressing the latches in the middle.
   b. Slide the DG8 cartridge into the right half of the holder, then drop it down.
   c. Press the halves of the holder together to snap it closed.

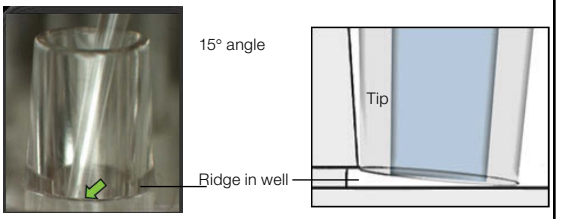
DG8 cartridge

Inserting the DG8 cartridge into the cartridge holder.

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

Air bubbles can cover the bottom of the well and result in 2,500–7,000 fewer droplets and poor data quality. They are difficult to see. To avoid creating air bubbles, use the following pipetting technique, which also ensures samples wet the bottoms of the wells so they are wicked into the microchannels (necessary for proper droplet generation).

- Use only 20 µl aerosol-barrier (filtered) Rainin pipet tips; do not use 200 µl pipet tips (see Table 1.2)
- Gently slide the pipet tip down the side of the well at a ~15° angle until it passes over the ridge near the bottom. Holding the 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
- After dispensing about half the sample, slowly draw the tip up the wall while dispensing the rest of the sample; do not push the pipet plunger past the first stop



Transferring sample to the sample wells (middle row) of the DG8 cartridge. Hold the pipet tip at a 15° angle and at the bottom of the well (middle and right panels); do not dispense sample onto the wall or side of the well.

3. Dispense the droplet generator (DG) oil in the reagent trough (see Table 2.1 for volumes required; see Table 1.2 for PCR supermix and DG oil compatability).

Reagent trough and droplet generator oil.

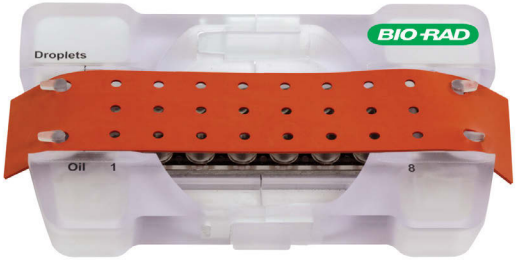
Table 2.1. Droplet generator (DG) oil requirements.

# Wells	Volme of Oil
8	700 µl
24	1,820 µl
48	3,500 µl
96	6,860 µl

4. Using a multichannel pipet, fill each oil well (bottom row) with 70 µl DG oil from the reagent trough.

Filling the oil wells with droplet generator oil.

5. Hook the gasket over the cartridge holder using the holes on both sides. The gasket must be securely hooked on both ends of the holder; otherwise, pressure sufficient for droplet generation will not be achieved.



Correct placement of the gasket over the cartridge holder.

6. Open the QX200 droplet generator by pressing the button on the green top and place the cartridge holder into the instrument. When the holder is in the correct position, both the power (left light) and holder (middle light) indicator lights are green (see Table 2.2)
7. Press the button on the top again to close the door. This initiates droplet generation: a manifold positions itself over the outlet wells, drawing oil and sample through the microfluidic channels, where droplets are created. Droplets flow to the droplet well, where they accumulate. The droplet indicator light (at right) flashes green after 10 sec to indicate droplet generation is in progress.

QX200 droplet generator with DG8 cartridge in place.

8. When droplet generation is complete, all three indicator lights are solid green. Open the door by pressing the button, and remove the holder (with DG8 cartridge still in place) from the unit. Remove the disposable gasket from the holder and discard it. The top wells of the cartridge contain droplets, and the middle and lower wells are nearly empty with a small amount of residual oil.

Keep the DG8 cartridge in the holder.

Table 2.2. Status indicator lights on the QX200 droplet generator. If the central LED flashes amber, the gasket is not placed on the holder correctly or is missing and no seal was made. If the right LED flashes amber, a process error occurred because the volume is too low in at least one well.

Solid green	Power on	DG8 cartridge holder in place	Run complete
Flashing green	—	—	Run in progress
Flashing amber	—	No seal; no gasket or empty well	Low volume in well
Off	Power off	No DG8 cartridge holder	Idle

2.3 Preparation for PCR

1. Pipet 40 µl of the contents of the top wells (the droplets) into a single column of a 96-well PCR plate.

Use the following pipetting techniques to avoid shearing or coalescing the droplets:

To aspirate droplets from the DG8 cartridge:

- Use an 8-channel manual L-50 pipet with 200 µl tips (not wide- or narrow-bore)
- 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, vertical into 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 bottoms of the wells
- Slowly draw 40 µl of droplets into the pipet tip (should take ~5 sec, and ~5 µl air is expected); do not aspirate >40 µl, as this causes air to percolate through the droplets
- Pipet slowly. Apply a stable resistive force to the plunger to draw and aspirate droplets smoothly into and out of pipet tips

To dispense droplets into the 96-well plate, position the pipet tip along the side of the well — near, but not at, the bottom of the well — and slowly dispense the droplets (~5 sec).

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.

Aspirating droplets from the DG8 cartridge.

Dispensing droplets into a 96-well PCR plate.

2. Seal the PCR plate with foil immediately after transferring droplets to avoid evaporation. Use pierceable foil plate seals that are compatible with the PX1™ PCR plate sealer and the needles in the QX200 droplet reader (for example, catalog #181-4040). Follow the instructions in the PX1 PCR Plate Sealer Instruction Manual (bulletin 10023997).
   a. Set the plate sealer temperature to 180°C and time to 5 sec.
   b. Touch the arrow to open the PX1 tray door. Position the support block on the tray with the 96-well side facing up. Place the 96-well plate onto the support block and ensure that all plate wells are aligned with the support block.
   c. Cover the 96-well plate with one sheet of pierceable foil seal. (The yellow label on the Bio-Rad heat seal bag identifies the sealing surface.) Do not attempt to place the frame over the foil-covered plate. The frame is only for use with other seals.
   d. Once the 96-well plate is secured on the support block and covered with the pierceable foil seal, touch the seal button. The tray will close and heat sealing will initiate.
   e. When heat sealing is complete, the PX1 door opens automatically. Remove the plate from the block for thermal cycling. Remove the block from the PX1.
   f. Check that all the wells in the plate are sealed; the depressions of the wells should be visible on the foil. Once sealed, the plate is ready for thermal cycling.
3. Once droplets are removed, press the latches on the DG8 cartridge holder to open it. Remove the empty DG8 cartridge and discard it.

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

PX1 PCR plate sealer (left) and a sealed 96-well plate (right).

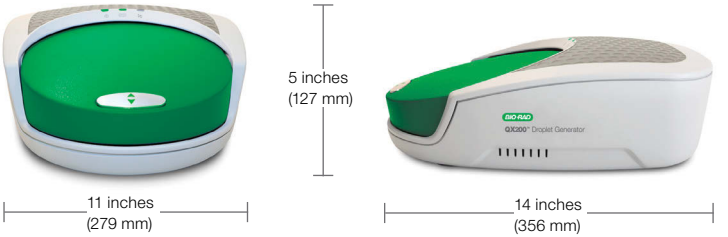
2.4 Subsequent Steps

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. When PCR amplification is complete, remove the 96-well plate from the thermal cycler and read the droplets using the QX200 droplet reader (follow the instructions in the QX200 Droplet Reader Instruction Manual, bulletin 10031906).

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, a set of eight wells in a single DG8 cartridge can be generated: four of these will be read after thermal cycling, and four will not be read. Refer to the QX200 Droplet Reader Instruction Manual (bulletin 10031906) for more details.

3 Specifications and Maintenance

3.1 Specifications



Weight	10 lb. (4.5 kg)
Size (W x D x H)	11 x 14 x 5" (28 x 36 x 13 cm)
Electrical requirements	100–240 V, 50/60 Hz, 60 W; voltage fluctuations not to exceed +10% of ratings
Temperature	15–30ºC
Altitude	0–6,560 ft (0–2,000 m)
Humidity	85% max (noncondensing)
Pollution degree	2 (indoor use)
Installation category	II (external power supply plugs into standard AC receptacle)
Ventilation requirement	5" (12 cm) left and right of machine and 10" (25 cm) behind should be unobstructed for proper ventilation

3.2 Maintenance

Surfaces of the instrument may require general cleaning. Use deionized/distilled water for general wipe down with a slightly dampened cloth. For decontamination, 10% bleach followed by 70% ethanol and/or deionized/distilled water may be used. Do not use acetone or tap water.

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

Apply standard MSDS (Material Safety Data Sheet) and OSHA practices when handling and disposing of generated waste.

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. These nonflammable fluids are inert and have low environmental impact and low toxicity. Collect waste in a polyethylene container and discard within one month.

Droplets made with Bio-Rad master mix have antimicrobial properties, but microbial growth is possible. The waste profile should contain the following: fluorinated hydrocarbons, water, fluorescent dye (from probes), intercalating dye, protein, and nucleic acids. The droplet generator is not intended to be used with biohazardous material.

Appendix A
Ordering Information

QX200™ ddPCR™ System

Catalog #	Description
186-4001	**QX200™ Droplet Digital™ PCR System**, includes droplet generator, droplet reader, laptop computer, software, associated component consumables
186-4002	**QX200 Droplet Generator**, includes droplet generator, 1 box of 24 cartridges, 1 pkg of 24 gaskets, 2 cartridge holders, 1 power cord
186-4003	**QX200 Droplet Reader**, includes droplet reader, ddPCR manual, 2 plate holders, USB cable, power cord
186-4007	**Droplet Generator Cartridges and Gaskets**, includes 5 pkg of 24 DG8™ cartridges, 5 pkg of 24 DG8 gaskets
186-4008	**DG8 Cartridges for QX100™/QX200 Droplet Generator**, 1 pkg of 24 cartridges
186-3009	**DG8 Gaskets for QX100/QX200 Droplet Generator**, 1 pkg of 24 gaskets
186-3051	**DG8 Cartridge Holder**
510-10608	**Droplet Reader Plate Holder**
186-3030	**Droplet Generator Oil for Probes**, 2 x 7 ml
186-3005	**Droplet Generator Oil for Probes**, 10 x 7 ml
186-4005	**Droplet Generator Oil for EvaGreen Dye**, 2 x 7 ml
186-4006	**Droplet Generator Oil for EvaGreen Dye**, 10 x 7 ml
186-3031	**Droplet Reader Oil**, 1 x 1 L
186-3004	**Droplet Reader Oil**, 2 x 1 L

ddPCR Reagents

186-3026	**ddPCR Supermix for Probes**, 2 ml (2 x 1 ml), 2x supermix
186-3010	**ddPCR Supermix for Probes**, 5 ml (5 x 1 ml), 2x supermix
186-3027	**ddPCR Supermix for Probes**, 25 ml (5 x 5 ml), 2x supermix
186-3028	**ddPCR Supermix for Probes**, 50 ml (10 x 5 ml), 2x supermix
186-3023	**Droplet PCR Supermix**, 2 ml (2 x 1 ml), 2x supermix
186-3024	**Droplet PCR Supermix**, 5 ml (5 x 1 ml), 2x supermix
186-3025	**Droplet PCR Supermix**, 25 ml (5 x 5 ml), 2x supermix

186-3021 **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

186-3022 **One-Step RT-ddPCR Kit for Probes**, 5 ml (5 x 1 ml), 500 x 20 µl reactions, 2x RT-ddPCR mix, includes 2 manganese acetate tubes

186-4033 **QX200™ ddPCR™ EvaGreen® Supermix**, 2 ml (2 x 1 ml), 200 x 20 µl reactions

186-4034 **QX200 ddPCR EvaGreen Supermix**, 5 ml (5 x 1 ml), 500 x 20 µl reactions

186-4035 **QX200 ddPCR EvaGreen Supermix**, 25 ml (5 x 5 ml), 2,500 x 20 µl reactions

186-4036 **QX200 ddPCR EvaGreen Supermix**, 50 ml (10 x 5 ml), 5,000 x 20 µl reactions

186-3052 **ddPCR Buffer Control Kit for Probes**, 2 x 4.5 ml bottles, 2x buffer

186-4052 **ddPCR Buffer Control Kit for EvaGreen**, 2 x 4.5 ml bottles, 2x buffer

Thermal Cycler and Plate Sealer

185-1196 **C1000 Touch™ Thermal Cycler with 96-Well Fast Reaction Module**, includes C1000 Touch thermal cycler chassis, 96-well fast reaction module, USB flash drive

181-4000 **PX1™ Plate Sealer**, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord

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