

QX100™ Droplet Generator

Instruction Manual

Catalog #186-3002



BIO-RAD

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Table of Contents

QX100™ Droplet Generator	1
1.1 Introduction	1
1.2 QX100 Droplet Generator	2
1.3 Installation and General Operation	3
Droplet Generation	5
2.1 Sample Preparation	5
2.2 Operation of the QX100 Droplet Generator	6
2.3 Preparation for PCR	10
Specifications and Maintenance	13
3.1 Specifications	13
3.2 Maintenance	14
Appendix A Ordering Information	15

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

1

QX100™ Droplet Generator

1.1 Introduction

The QX100™ Droplet Digital™ PCR (ddPCR™) system performs digital PCR with unrivaled accuracy and precision. The system consists of two instruments, the QX100 droplet generator and the QX100 droplet reader, and their associated consumables. The QX100 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 QX100 droplet reader. PCR-positive and PCR-negative droplets are counted to provide absolute quantification of target DNA in digital form.

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
- Mutation detection
- Gene expression analysis
- miRNA analysis
- Next-generation sequencing sample quantification

This manual covers use of the QX100 droplet generator and preparation for PCR. For information on the QX100 droplet reader, please refer to bulletin 10026321.

1.2 QX100 Droplet Generator

The QX100 droplet generator uses microfluidics to combine oil and water (sample) to create the droplets required for ddPCR analysis. It processes up to eight samples at a time, in under 2 minutes.

Following sample preparation using either the ddPCR supermix or One-Step RT-ddPCR kit for probes, 20 μ l each of eight prepared samples (or blanks) and droplet generator oil are transferred to the droplet generator (DG™) cartridge. The loaded cartridge is covered with a gasket and placed in the QX100 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 cyclor.

When cycling is complete, the plate is loaded into the QX100 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 (-).

The QX100 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 QX100 Droplet Reader Instruction Manual (bulletin 10026321).

Table 1.1. QX100 droplet generator components. Catalog # refers to replacement items (quantities may be different).

Component	Description	Catalog #
QX100 droplet generator	Instrument used for droplet generation	186-3002
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	186-3008
Droplet generator cartridge holders (2)	Position and hold the droplet generator cartridge in the instrument for droplet generation	186-3051
Power cord	Connects QX100 droplet generator to power source	Call technical support



QX100 droplet generator.

Table 1.2. Additional materials required for droplet generation.

Component	Recommended	Catalog #
PCR supermix (required)	ddPCR supermix for probes One-Step RT-ddPCR kit for probes	186-3010, 186-3026, 186-3027, 186-3028 186-3021, 186-3022
Control	Buffer control kit	186-3052
Droplet generator oil	Droplet generator oil (10 x 7 ml)	186-3005
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
Pipet tips	Filtered	Rainin GP-L10F, GP-L200F
96-well PCR plates	twin.tec semi-skirted 96-well plate	Eppendorf 951020362
Reagent trough	For droplet generator oil	Thermo 95128095
Foil plate seals	Easy Pierce foil plate seals	Thermo AB-0757
Plate sealer		Eppendorf 951023078
8-cap strips	Any	

1.3 Installation and General Operation

- Connect the QX100 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) space clear 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, and centrifuge briefly.

- The concentration of intact human genomic DNA should be <66 ng per 20 μ l reaction. If using higher concentrations, digest DNA with a restriction endonuclease that does not cut target or reference amplicons
- Use either the ddPCR™ supermix for probes or the One-Step RT-PCR kit for probes, which contain reagents required for droplet generation. Follow the 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 minutes) 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, clean pipets, work in a clean area such as a PCR hood, and use low protein binding tubes

2.2 Operation of the QX100™ Droplet Generator

The QX100 droplet generator prepares droplets for eight samples at a time. Droplet generation takes 2 minutes for each set of eight samples (20–30 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 the DG8 cartridge with sample or oil unless it is already 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



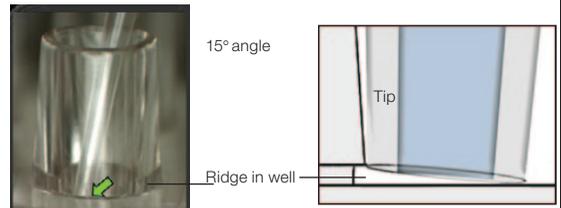
Inserting the DG8 cartridge into the cartridge holder.

3. 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 techniques, which also ensure 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 $\sim 15^\circ$ 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.

4. Dispense the droplet generator (DG) oil in the reagent trough. Use 700 μl DG oil per 8 samples (one DG oil bottle contains enough oil for a 96-well PCR plate).



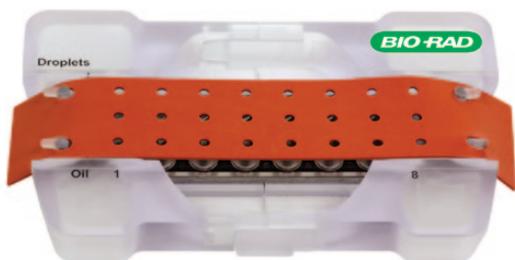
Reagent trough and droplet generator oil.

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



Filling the oil wells with droplet generator oil.

- 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, sufficient pressure will not build up for droplet generation.



Correct placement of the gasket over the cartridge holder.

- Place the cartridge holder into the QX100 droplet generator and initiate droplet generation:
 - Open the instrument by pressing the button on the green top
 - 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 (Table 2.1)
- Press the button on the top again to close door. This initiates droplet generation: a manifold positions itself over the outlet wells, drawing oil and sample through 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.

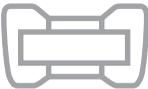


QX100 droplet generator with DG8 cartridge in place.

9. When droplet generation is complete, all three indicator lights are solid green. Open the door and remove the holder (with DG8 cartridge still in place) from the unit. Remove the disposable gasket from the holder and discard. The top wells now 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 2.1. Status indicator lights on the QX100 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 gasket; empty well or seal	Low volume in well
Off	Power off	No DG8 cartridge holder	Idle

2.3 Preparation for PCR

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



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

To aspirate droplets from the DG8 cartridge:

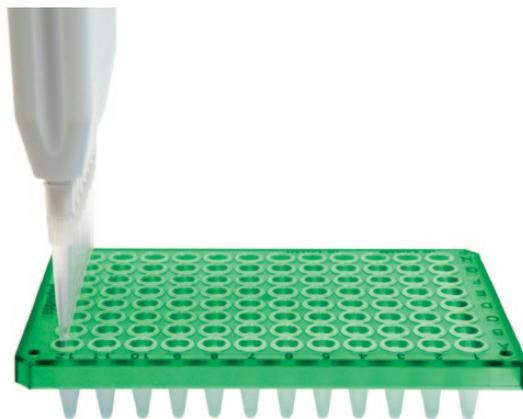
- Use an 8-channel manual P-50 Pipetman with 200 μ l tips (not wide- or narrow-bore); do not use a P-20 or P-1000 Pipetman
- Place the cartridge holder on a flat surface and position the pipet tips in each of the 8 top wells at a $\sim 30\text{--}45^\circ$ angle, vertical into the junction where the 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 μ l droplets into the pipet tip (should take ~ 5 sec, and ~ 5 μ l air is expected); do not aspirate >40 μ l, 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).

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



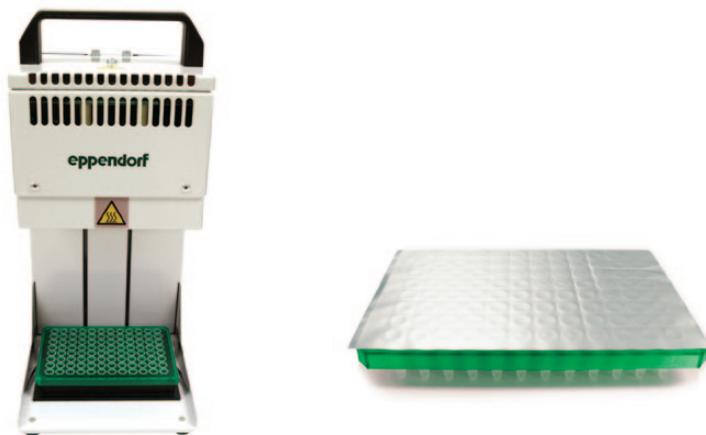
Aspirating droplets from the DG8 cartridge.



Dispensing droplets into a 96-well PCR plate.

2. Seal the PCR plate wells with foil immediately after transferring droplets to avoid evaporation. Use Easy Pierce foil plate seals (compatible with the needles in the QX100 droplet reader) and a compatible thermal plate sealer.
 - a. Allow the plate sealer to warm up for at least 10 min before use.
 - b. Place the foil seal over the plate and place the plate in the plate sealer.
 - c. Press the sealer firmly against plate for 3 sec, then rotate the plate 180° and seal for another 3 sec.
 - d. 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.

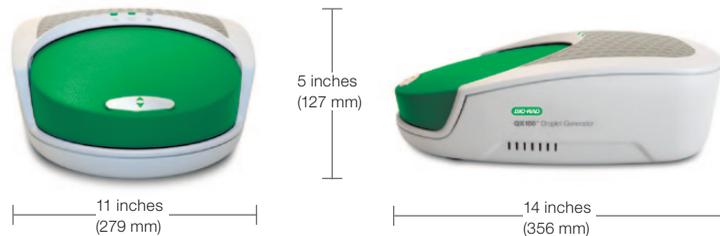


Eppendorf plate sealer (left) and a sealed 96-well plate (right).

3

Specifications and Maintenance

3.1 Specifications



Weight	10 lbs. (4.5 kg)
Size (W x D x H)	28 x 36 x 13 cm (11 x 14 x 5")
Electrical requirements	100–240 V, 50/60 Hz 60 W; voltage fluctuations not to exceed +10% of ratings
Temperature	15–30°C
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 dispose 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), protein, and nucleic acids. The droplet generator is not intended to be used with biohazardous material.

Appendix A

Ordering Information

QX100™ ddPCR™ System

Catalog #	Description
186-3001	QX100™ Droplet Digital™ PCR System , includes droplet generator, droplet reader, laptop computer, software, associated component consumables
186-3002	QX100 Droplet Generator , includes droplet generator, 1 box of 24 cartridges, 1 pkg of 24 gaskets, 2 cartridge holders, 1 power cord
186-3003	QX100 Droplet Reader , includes droplet reader, ddPCR manual, 2 plate holders, USB cable, power cord
186-3005	Droplet Generator Oil , 10 x 7 ml bottles
186-3004	Droplet Reader Oil , 2 x 1 L bottles
186-3006	Droplet Generator Cartridges and Gaskets , includes 5 pkg of 24 DG8™ cartridges, 5 pkg of 24 DG8 gaskets
186-3008	DG8 Cartridges for QX100 Droplet Generator , 1 pkg of 24 cartridges
186-3009	DG8 Gaskets for QX100 Droplet Generator , 1 pkg of 24 gaskets
510-10608	Droplet Reader Plate Holder

ddPCR Reagents

186-3010	ddPCR Supermix for Probes , 5 x 1 ml tubes 2x supermix, for use in sample preparation for droplet generator in the QX100 Droplet Digital PCR system
186-3026	ddPCR Supermix for Probes , 2 x 1 ml tubes
186-3027	ddPCR Supermix for Probes , 5 x 5 ml tubes
186-3028	ddPCR Supermix for Probes , 10 x 5 ml tubes
186-3021	One-Step RT-ddPCR Kit for Probes , 2 x 1 ml, 200 x 20 µl reactions, 2x RT-ddPCR mix, for use with QX100 Droplet Digital PCR system; contains manganese acetate
186-3022	One-Step RT-ddPCR Kit for Probes , 5 x 1 ml, 500 x 20 µl reactions, 2x RT-ddPCR mix, for use with QX100 Droplet Digital PCR system; contains manganese acetate
186-3052	Buffer Control Kit , 2 x 4.5 ml bottles 2x buffer, for use with QX100 Droplet Digital PCR system

Thermal Cyclers

- 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
- 186-1096 **T100™ Thermal Cycler**, includes 96-well thermal cycler, power cord, tube support ring



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