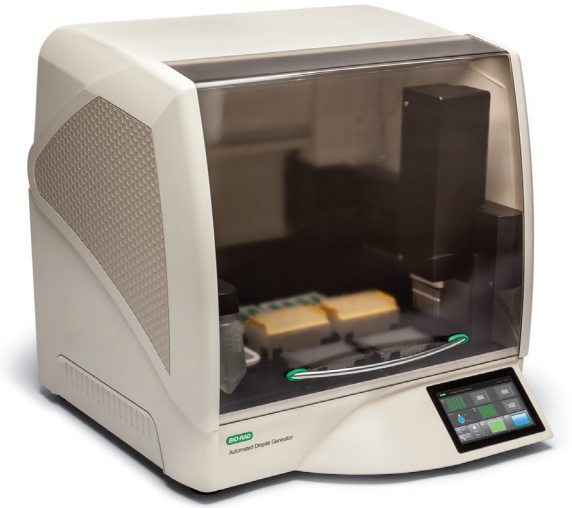
Automated Droplet Generator, IVD

Instruction Manual

REF 12001630
17002229

Manual revision: A

CE IVD



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Bio-Rad Technical Support

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

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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 61010-1:2010 (3rd ed.), EN61010-1:2010 (3rd 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
- UL 61010-1:2004, Laboratory equipment, Test & Measurement Equipment and Industrial Process Controls
- CAN/CSA 22.2 No 61010-1-04, Safety Requirements for Electrical. Equipment for Measurement, Control, and Laboratory Use, Part I: 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 European Directive for in-vitro diagnastic medical devices 98/79/EC.

The cTUV mark indicates that the product has been tested to TUV 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.

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.

This instrument is for use only by trained personnel.

Do not position the equipment so that it is difficult to operate the plug of the power supply. The plug of the power supply is the disconnect device.

No serviceable parts inside.

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 adapter 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

Transporting the Instrument

Avoid dropping or subjecting the instrument to shock when transporting.

This product weighs in excess of 100 lb (45.4 kg). When lifting, two people are required. Grip from the underside, one person on each opposite side. After unpacking for placement of equipment, transport per lifting instructions; place near grounded outlet with plug of power supply accessible.

Biohazards

If biohazardous samples are present, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location.

General Precautions

- Always wear laboratory gloves, coats, 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
- Remove wristwatches and jewelry before working at the bench
- Store all infectious or potentially infectious material in unbreakable leak-proof containers
- Before leaving the laboratory, remove protective clothing
- 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
- Change gloves frequently. Remove gloves immediately when they are visibly contaminated
- Do not expose materials that cannot be properly decontaminated to potentially infectious material
- Upon completion of the operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant (for example, a 1:10 dilution of household bleach)
- No biohazardous substances are exhausted during normal operations of this instrument

Table of Contents

1 QX200™ AutoDG™ ddPCR™ System, IVD

1.1 Introduction

Bio-Rad's QX200 AutoDG ddPCR System, IVD combines water-oil emulsion droplet technology with microfluidics to perform accurate and precise digital PCR. The system consists of two instruments — an Automated Droplet Generator, IVD and a QX200 Droplet Reader, IVD — and associated consumables. The Automated Droplet Generator, IVD partitions each sample into ~20,000 uniform nanoliter-sized droplets in which nucleic acid molecules are distributed in a random fashion. Each droplet serves to partition the reactions. The 96-well PCR plate of droplets is removed from the AutoDG instrument, sealed, and PCR is performed to end point in a thermal cycler. Droplets from each sample are analyzed individually on the QX200 Droplet Reader, IVD; the droplets stream single file through the reader for fluorescence analysis.

PCR-positive and PCR-negative droplets are counted to provide absolute quantification of target DNA in digital form. Positive droplets, which contain at least one copy of the target DNA or RNA molecule, exhibit increased fluorescence compared to negative droplets. The fraction of PCR-positive droplets enables the target to be quantified according to the Poisson distribution. Alternatively, amplified products can be extracted from droplets following PCR for downstream applications, such as sequencing or cloning.

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

The ddPCR System lets you:

- Achieve absolute quantification without the use of a standard curve
- Detect rare DNA target copies with unmatched sensitivity
- Determine copy number variation with unrivaled accuracy
- Measure gene expression levels with precision
- Design scalable assays for high sensitivity or high throughput
- Expand applications using flexible ddPCR chemistry — EvaGreen or probes

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 Automated Droplet Generator, IVD and preparation for PCR. For information on the QX200 Droplet Reader, IVD, please refer to the QX200 Droplet Reader, IVD Instruction Manual, part number 10000044967.

1.2 System Components

Bio-Rad's Automated Droplet Generator, IVD simplifies the Droplet Digital PCR workflow, making digital PCR both scalable and practical. The AutoDG instrument prepares droplets for up to 96 samples at a time with minimal hands-on time required. In less than 45 minutes, a plate of droplets can be generated. The plate can then be sealed, thermal cycled, and ddPCR analysis can be performed on a QX200 Droplet Reader, IVD.

Automated droplet generation eliminates user-to-user variability that can be associated with manual droplet generation. The AutoDG instrument's HEPA-filtered enclosure reduces contamination during droplet generation. Consumables are automation-friendly with SBS standard access.

The system's guided quick load allows users to get started faster. The large, color touch screen provides simple setup and plate tracking. Select the desired number of columns across a 96-well plate, load Droplet Generation Oil and only the consumables you need as prompted by the system, and start the run. A countdown timer will display the time remaining so users can maximize their time in the lab. When the plate is ready, users are notified and a time-elapsed counter begins. Every run is stored in an exportable log file for future reference.

Following reaction preparation using ddPCR supermix, 22 µl of each of up to 96 prepared samples (or blanks) in a 96-well plate are loaded into the Automated Droplet Generator, IVD. A bottle of the Automated Droplet Generation Oil is loaded into the instrument, along with DG32™ Cartridges and Pipet Tips for the AutoDG instrument. 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 automated droplet generation, the 96-well PCR plate of droplets is sealed, and PCR is performed to end point in a thermal cycler.

When cycling is complete, the plate is loaded into the QX200 Droplet Reader, IVD. 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 quantification of droplets is not required, PCR products can be extracted from droplets following thermal cycling for downstream applications, such as sequencing or cloning.

The Automated Droplet Generator, IVD includes the components listed in Table 1. Additional requirements for the Droplet Digital PCR workflow (automated droplet generation, sealing, cycling, and reading) are listed in Table 2.

Table 1. Automated Droplet Generator, IVD components. Items shipped with the Automated Droplet Generator, IVD (catalog #12001630).

Component	Description	Catalog #
Automated Droplet Generator, IVD	Instrument used for Automatic Droplet Generation	12001630
Power cord	Connects Automated Droplet Generator, IVD to power source	Call technical support
Cooling block accessory	Prevents evaporation during droplet generation	12002819
Oil waste reservoir	Collects oil waste from priming and flushing	Call technical support

Table 2. Additional materials required for Droplet Digital PCR.

Component	Description	Catalog #
Automated Droplet Generator, IVD		
ddPCR Dx AutoDG Consumable Pack	Includes 3 x 5 ddPCR 96-Well Plates, 1 x 50 ddPCR Pierceable Foil Heat Seals, 1 x 50 ml Automated Droplet Generation Oil for Probes, 1 x 15 DG32 Cartridges, 10 x 96 Pipet Tips, and instructions for use required for automated droplet generation	12001922
ddPCR Dx AutoDG Supermix Pack	Includes 5 x 1 ml supermix required for Droplet Digital PCR	12003031
Buffers	QX200 Buffer Control Kit for EvaGreen	186-4052
	QX200 Buffer Control Kit for Probes	186-3052
Sealer and Thermal Cycler		
Plate Sealer	PX1™ PCR Plate Sealer	181-4000
Thermal Cycler	C1000 Touch™ Thermal Cycler with 96-deep well real-time PCR reaction module	184-1000-IVD
QX200 Droplet Reader, IVD*		
ddPCR Dx Droplet Reader Oil Pack	Includes 1 x 1000 ml Droplet Reader Oil required for Droplet Digital PCR	12002526

* For information on components shipped with QX200 Droplet Reader, see part number 10000044967.

1.3 Installation and General Operation

Automated Droplet Digital PCR involves the following steps (4.5–5 hours for the complete workflow):

1. Prepare PCR-ready samples — combine nucleic acid sample (DNA or RNA), primers, and probes (FAM, VIC, or HEX) or intercalating dye (EvaGreen) with Bio-Rad ddPCR supermix (see Table 2).

2. Make droplets — load 22 µl of the ddPCR reaction into a 96-well PCR plate, then load the plate and required consumables into the Automated Droplet Generator, IVD to partition the sample into droplets. The Automated Droplet Generator, IVD uses microfluidics to combine oil and aqueous sample to generate the nanoliter-sized droplets required for ddPCR analysis. It processes up to 96 samples at a time in less than 45 min.

3. Perform PCR — remove the 96-well PCR plate containing droplets from the Automated Droplet Generator, IVD, seal the plate with foil, and perform PCR to end point (~40 cycles) using a PX1™ Plate Sealer and C1000 Touch™ Thermal Cycler (see Table 2).

4. Read droplets — load the plate into the QX200 Droplet Reader, IVD and start your run. The droplet reader sips each sample, singulates the droplets, and streams them in single file past a two-color detector. The detector reads the droplets to determine which contain a target (+) and which do not (–).

   If reading or quantifying droplets and recovering material from droplets in parallel, prepare two sets of reactions, one for each application. For example, a set of eight wells of droplets can be generated: four of these will be read after thermal cycling, and four will not be read.

5. Analyze results — the droplet reader connects to a laptop computer running QuantaSoft™ Software. The software provides a complete set of tools for setting up and naming samples, running and controlling the instrument, and analyzing results. It also reads the positive and negative droplets in each sample and plots the fluorescence, droplet by droplet. The fraction of positive droplets in a sample determines the concentration of target in copies/µl.

The QX200 ddPCR System is compatible with hydrolysis probe (TaqMan) chemistry and can detect up to two fluorophores and up to four targets at a time (FAM/VIC or FAM/HEX). It is also compatible with EvaGreen chemistry. Use only the approved Bio-Rad supermixes listed in Table 2 with this system; using unapproved supermixes may harm the instrument and voids the warranty.

1.4 System Setup and General Operation Instructions

Connect the Automated Droplet Generator, IVD to a power source using the power cord and power adapter provided. Leave 2" (~5 cm) clear space behind and 5" (~13 cm) clear to the right and left of the instrument for proper ventilation. Position the instrument such that it can be easily disconnected from the power source, should that become necessary for servicing the equipment.

The Automated Droplet Generator, IVD is powered on by plugging it into a power source.

2 Using the Automated Droplet Generator, IVD

2.1 Powering On the Automated Droplet Generator, IVD

The Automated Droplet Generator, IVD (AutoDG™) is designed to remain powered on in order to preserve positive airflow inside of the instrument and track consumable use. The instrument stays in an idle state when not being used. Please see Section 2.2 for instructions on starting a run from idle mode.

1. When powering on the Automated Droplet Generator, IVD, you will see a startup screen while the instrument powers on and performs a self-check. Please note that the door will automatically close.

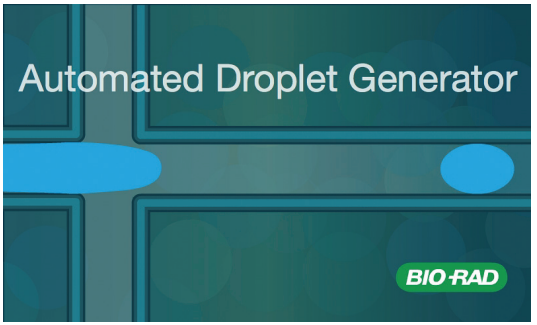


Fig. 1. Startup screen when instrument is powered on.

2. If the instrument deck is empty, the indicator lights on the deck of the Automated Droplet Generator, IVD should be off, indicating that no consumables are present. The corresponding areas of the touch screen will be gray.

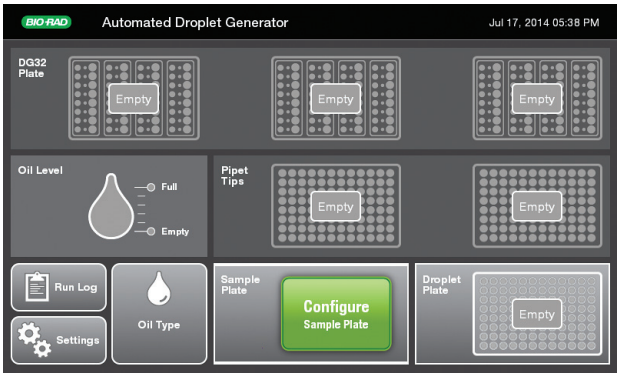
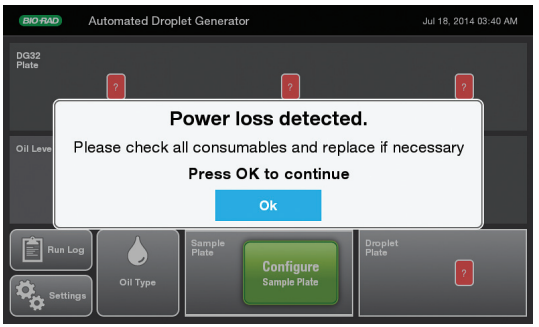


Fig. 2. Home screen, no consumables loaded.

If consumables were present in the instrument when power was lost or disconnected, an error message will appear prompting you to check and reset the consumables if necessary. The cartridges, gaskets, tips, and plates are all single-use consumables and should be discarded after use. If the consumables in the instrument are of questionable use after power loss, please remove and discard. Proceed to Section 2.2 to start a run on the AutoDG instrument.

A



B

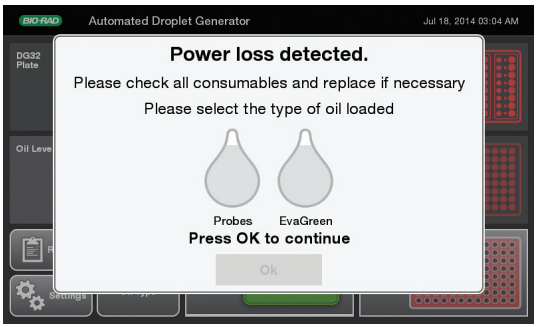
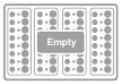
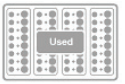
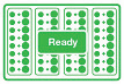
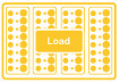
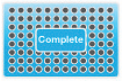
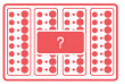


Fig. 3. Error messages displayed after power loss. A, when cartridges, tips, and/or plates are detected after power loss; **B**, when cartridges, tips, and/or plates as well as a bottle of oil are detected after power loss.

2.2 Starting a Run on the AutoDG Instrument

1. Bring the Automated Droplet Generator, IVD out of idle mode by touching the screen.
2. Check the indicator lights on the deck of the AutoDG instrument nd the consumable icons on the touch screen. Table 3 explains the deck lighting and touch screen icons and the instrument status they indicate.

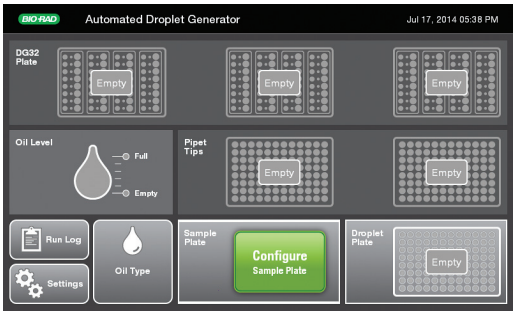
Table 3. AutoDG instrument status as indicated by deck lighting and touch screen.

Deck Lighting Status	Touch Screen Icon Status		Indication
Off	Gray, Empty		Ready to configure a new run
Off	Gray, Used		Ready to configure a new run; instrument will prompt for consumable replacement in used positions when the next run is configured
Green	Green, Ready		Ready to configure a new run; consumables in the green positions are ready to be used
Yellow	Yellow, Load		Run configured, load consumables as prompted (this status occurs only during run setup)
Blue	Blue, Complete		Run complete and droplets ready; occurs only at droplet plate position
Red	Red, ?		Consumable status unknown after power loss, please confirm manually

The Automated Droplet Generator, IVD accepts either a sealed or open 96-well PCR plate containing 22 µl prepared ddPCR™ reactions. Detailed sample preparation and reaction setup information can be found in the ddPCR Supermix product inserts.

3. To create a plate of droplets with the Automated Droplet Generator, IVD:
   a. Touch the **Configure Sample Plate** button at the bottom center of the screen.
   b. Touch or swipe across the screen to select the columns in which your samples are located on the sample plate. Touching a selected column deselects it. You can touch any orientation of columns.
   c. The plate name and plate notes are optional; touching the fields will bring up a keyboard on the screen. Click **OK** when done.

A



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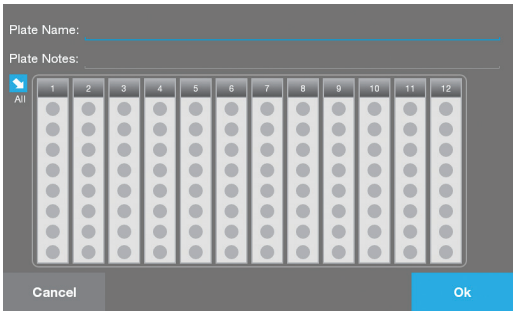
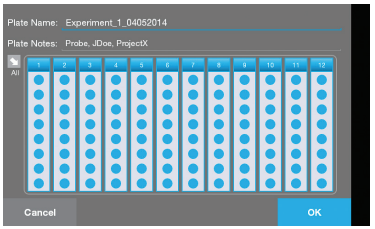
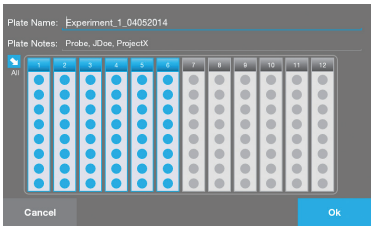


Fig. 4. A, Home screen; B, Configure Sample Plate screen.

A



B



C

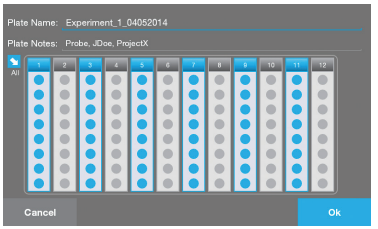


Fig. 5. Configure Sample Plate screen with A, full plate of samples selected; B, half plate of samples selected; C, alternating columns of samples selected.

4. Based on the number of columns selected in the previous step, the consumable icons on the screen will begin to blink yellow to indicate where new consumables need to be loaded into the instrument.
   - If the blinking yellow icon displays **Load** on the screen, remove the previously used consumable (if applicable) and load a new consumable into the designated area of the instrument.
   - If the icon remains gray on the screen, that consumable is not needed to complete the currently configured run.

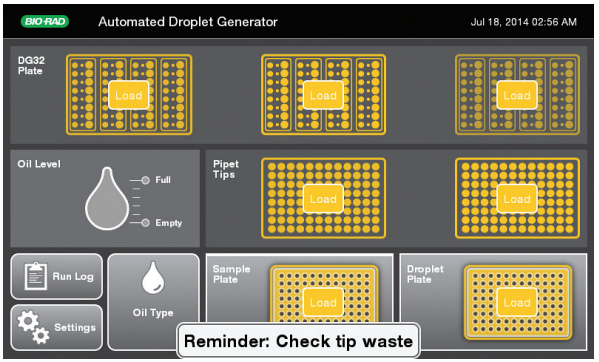


Fig. 6. The Automated Droplet Generator, IVD keeps track of the consumables so long as it is connected to a power supply. Based on the number of columns configured, the system will prompt for only the number of consumables that need to be loaded to complete the run. In this example, a full plate has been selected.

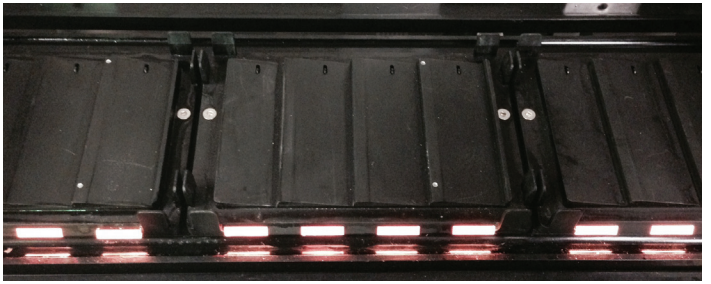
5. Open the door on the AutoDG instrument by lifting up on the handle at the front of the instrument. The electronic braking system will assist you in this task and prevent the lid from closing accidentally.

> The Automated Droplet Generator, IVD door will close to preserve HEPA-filtered enclosure if left open for longer than 20 minutes while in idle mode (not generating droplets). There is an audible click when the door brake releases, and the door closes slowly. Please observe the pinch points of the instrument and keep hands clear.

To avoid contamination, load the consumables from the back to the front of the instrument. Nothing should be placed on the instrument deck outside of the dedicated consumable holders.

6. To load the DG32™ AutoDG Cartridges along the back row of the instrument:
   a. Remove the plastic wrapping from the DG32 cartridges and place, with the green gaskets to the right, into the three plate holders. The holders are keyed for proper orientation of each DG32 cartridge to prevent incorrect loading.
   b. The lights on the DG32 plate holders will change from yellow to green when the DG32 cartridges are inserted correctly. If a light remains yellow, try repositioning the plate in a different orientation.
   c. As the lights turn green on the deck, the corresponding icons on the touch screen will go from blinking yellow to solid green, and **Ready** will be displayed.

A



B

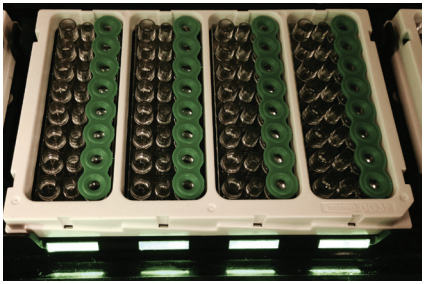


Fig. 7. A, DG32 Cartridge holders on the AutoDG deck in the "Load" state, indicated by yellow lights; B, green lights indicate the DG32 Cartridge has been correctly loaded and is ready.

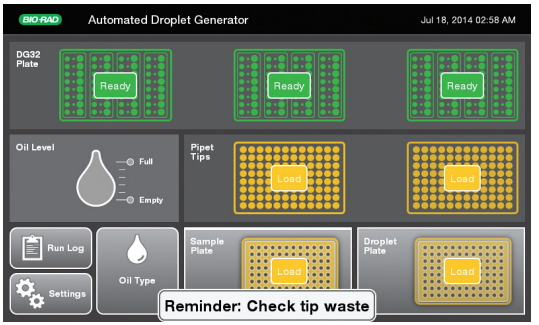


Fig. 8. DG32 consumables have been correctly loaded. The **Ready** status appears on the DG32 icons on the screen.

7. To load the AutoDG Pipet Tips along the center row of the instrument:
   a. Remove the plastic wrapping and box lids from the tip boxes and place into the plate holders in the middle of the deck. Only full tip boxes should be loaded.
   b. Remove the tip waste bin containing any tips from a previous run and replace with a clean waste bin.
   c. The lights on the tip box holders will change from yellow to green when the tip boxes are inserted correctly.
   d. As the lights turn green on the deck, the corresponding areas of the touch screen will go from blinking yellow to solid green, and **Ready** will be displayed.

Please note that only AutoDG Pipet Tips should be used; other tips can damage the instrument.

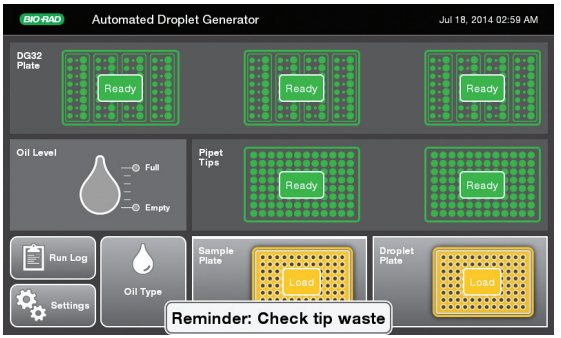


Fig. 9. Pipet tip boxes have been correctly loaded. The **Ready** status appears on the Pipet Tip icons on the screen.

8. To load the 96-well PCR plate containing your prepared ddPCR reactions into the front row of the instrument:
   a. The sample plate can be sealed with a PX1™ Plate Sealer and heat-sealing foil in advance of loading into the AutoDG instrument. Each well should contain 22 µl of your prepared ddPCR reaction. Please see the supermix product insert for more detailed information on ddPCR reaction setup.
   b. Place the plate into the front left plate holder, labeled on the screen as **Sample Plate**. The holder is keyed for proper orientation and contains plate clips to support sealed plates.
   c. The light on the Sample Plate holder will change from yellow to green when the plate is inserted correctly. If the light remains yellow, try repositioning the plate in a different orientation.
   d. As the light turns green on the deck, the corresponding icon on the touch screen will go from blinking yellow to solid green, and **Ready** will be displayed.

Please note that a sample plate is required for every run, regardless of the number of columns selected.

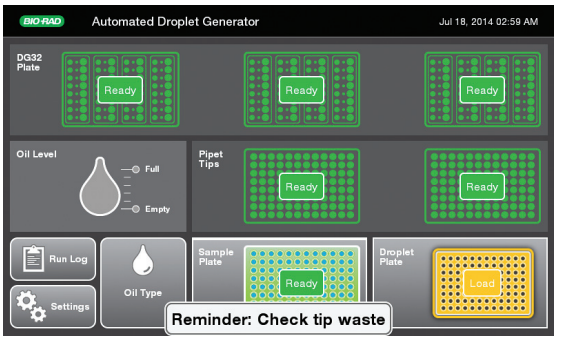


Fig. 10. Sample plate has been correctly loaded. The **Ready** status appears on the Sample Plate icon on the screen.

The cooling block should be placed in a –20°C freezer for at least 2 hours before configuring a run on the AutoDG nstrument and inserting the Droplet Plate assembly into the instrument. The block goes from a pink color at room temperature to a dark purple color when properly cooled.

9. To load the Droplet Plate assembly:
   a. Remove the cooling block from the freezer and place into the front right plate holder, labeled on the screen as **Droplet Plate**. The holder is keyed for proper orientation of the cooling block. The block should be a dark purple color, indicating it is at the proper temperature. If the block is pink, it has warmed up and should not be used (Figure 12).
   b. The light on the instrument will change from yellow to green when the block is inserted correctly. If the light remains yellow, try repositioning the block in a different orientation.
   c. As the light turns green on the deck, the corresponding area of the screen will go from blinking yellow to solid green, and **Ready** will be displayed.
   d. Place a clean 96-well PCR plate for droplet collection into the cooling block accessory. The cooling block is also keyed for proper orientation of the plate.

Please note that a clean droplet plate is required for every run, regardless of the number of columns selected. Once generated, the droplets will be dispensed into the same plate orientation as the ddPCR reactions were taken from the sample plate.

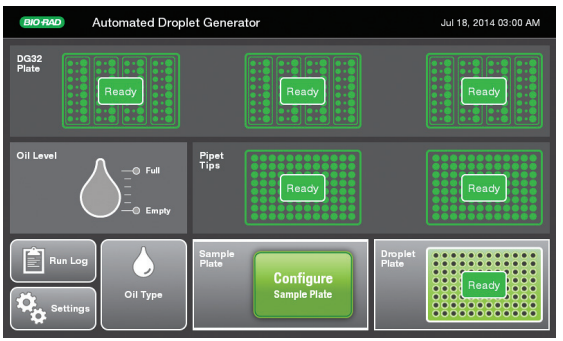


Fig. 11. Droplet plate and cooling block assembly have been correctly loaded. The **Ready** status appears on the Droplet Plate icon on the screen.

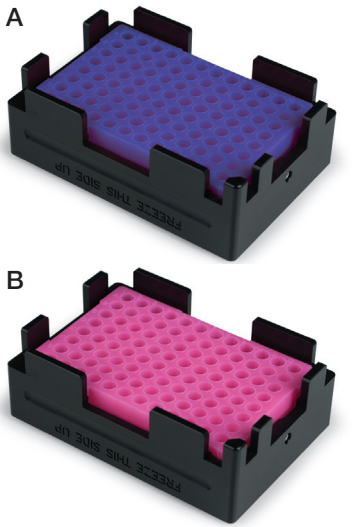


Fig. 12. A, cooling block assembly is purple, indicating it is ready to use for droplet generation. B, Cooling block assembly is pink, indicating it should be placed in the freezer before use.

Why do I need a cooling block?

The AutoDG instrument uses a cooling method to prevent droplet evaporation, much like the Droplet Generator requires you to cover the wells once droplets are transferred.

10. To load Automated Droplet Generation Oil into the instrument:
    a. Remove the cap from the bottle of Automated Droplet Generation Oil and twist the bottle into the tower of the oil delivery system at the front left corner of the instrument. Turn the bottle until it does not move; the label on the bottle should face out.
    b. Select the type of Automated Droplet Generation Oil that was loaded into the instrument by touching the droplet for either **Probes** or **EvaGreen** oil. The droplet you select will turn blue; touch **OK** to set the oil type.
    c. The Oil Level icon on the screen will turn blue and display the current oil level of the bottle. The system will display the oil type at the bottom left of the screen as well.

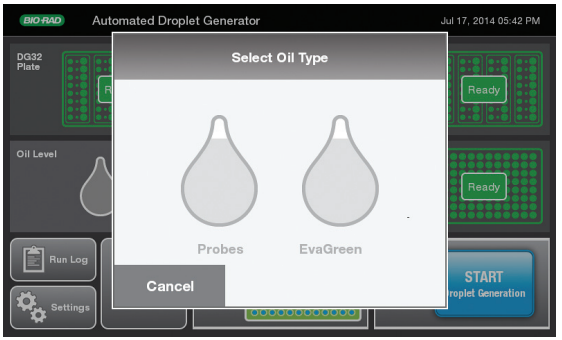


Fig. 13. Select oil type when loading a bottle of Automated Droplet Generation Oil.

If a bottle of Automated Droplet Generation Oil was previously loaded, you may be prompted to confirm the type of oil currently loaded into the instrument.

If the last plate was run on the AutoDG instrument with a different oil type than the one being currently loaded and selected, the instrument will perform a small volume purge of the oil through the delivery system and into the oil waste reservoir. The total run time will be a few minutes longer when this occurs, but droplet generation will not be impacted. To prevent this operation in the future, see Section 2.4 on advanced oil loading and switching.

Automated Droplet Generation Oil for EvaGreen should be used only in combination with the QX200™ ddPCR system and ddPCR EvaGreen Supermix. The use of other supermixes can lead to poor results and damage to the QX200 Droplet Reader, IVD. The QX100™ ddPCR System does not support EvaGreen chemistry; running this chemistry will damage the instrument.

11. Once all of the consumables are loaded and the corresponding lights are green on the deck and touch screen, a blue Start button will appear at the bottom right of the screen. Touching **Start** will bring up a confirmation window indicating the type of Droplet Generation Oil loaded, the number and orientation of columns selected for droplet generation, and any plate name and details entered during configuration of the sample plate.

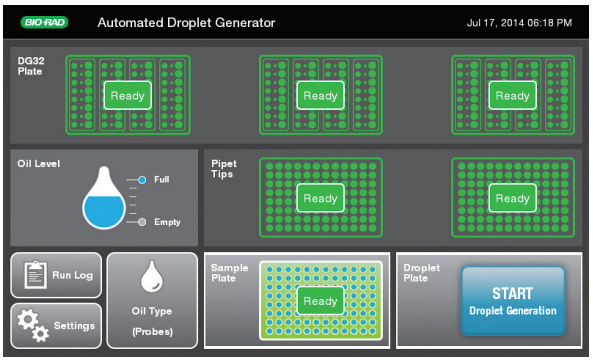
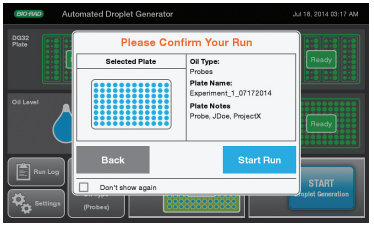
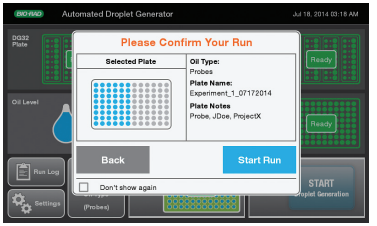


Fig. 14. Home screen; all consumables are correctly loaded and the system is ready to start droplet generation.

A



B



C

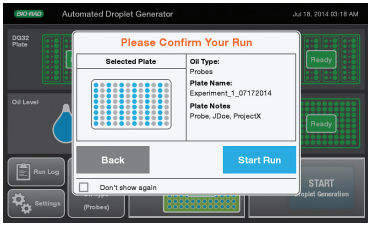


Fig. 15. Run confirmation screen with A, full plate of samples selected; B, half plate of samples selected; C, alternating columns of samples selected.

12. Once you have confirmed the plate setup, touch the **Start Run** button to begin droplet generation. The door will automatically close at the beginning of the run and must remain closed during the run. Opening the door before the **Droplets Ready** message appears may cause the instrument to terminate the run and samples to be lost. Please see step 13 below for information on aborting a run.

    If the selected columns on the confirmation screen do not match the location of samples in your sample plate, touch **Back** to change. Touch the sample plate to bring up the Configure Sample Plate window and change your selection.

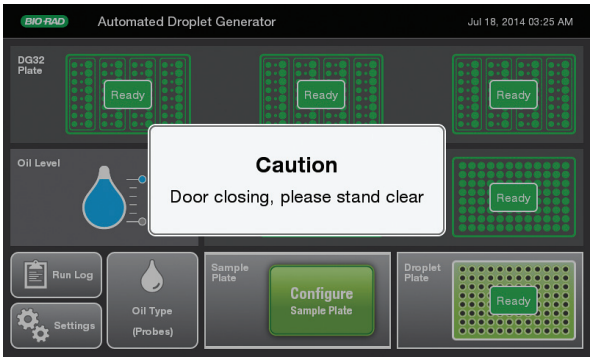
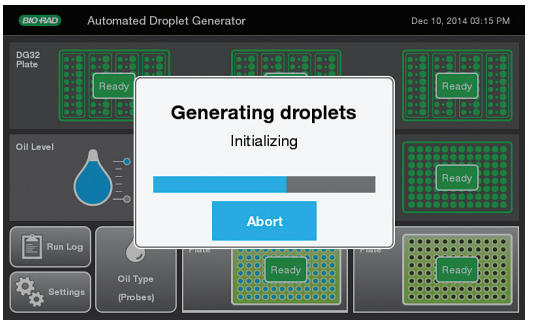


Fig. 16. The AutoDG door automatically closes at the start of each run and must remain closed until droplet generation is complete.

13. After a brief initialization, the Automated Droplet Generator, IVD will display a countdown timer on the screen with time remaining until the plate of droplets is ready. No additional action is needed until the plate is ready. The initialization can take 1–5 min, depending on whether the oil type has been changed or not (see Section 2.4).

A



B

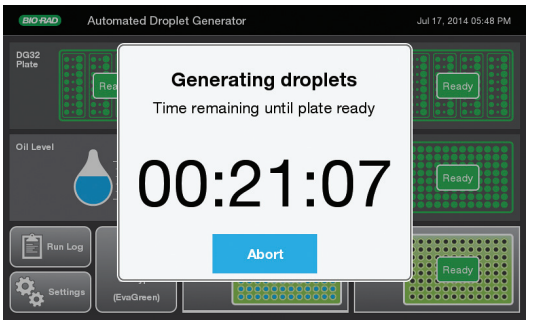
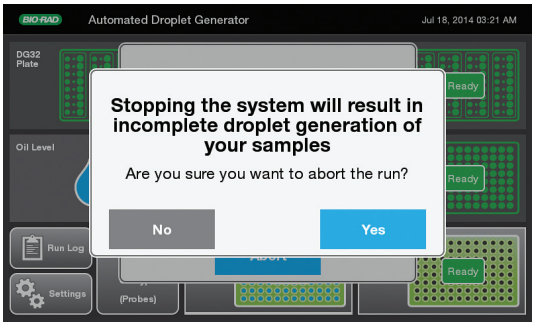


Fig. 17. A, initializing display with progress bar at the beginning of a run; B, countdown timer display indicates the time remaining until droplet generation is complete and the Droplet Plate can be removed.

If for any reason you need to stop the run, touch the **Abort** button on the countdown timer screen. A confirmation window will appear; touch **Yes** to abort the run or **No** to return to the countdown timer. Please note that stopping the Automated Droplet Generator, IVD during a run can result in loss of the current column of samples being processed.

A



B

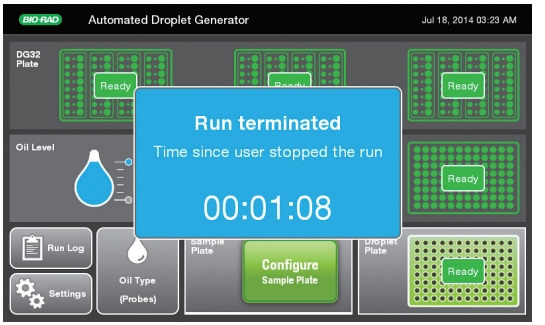
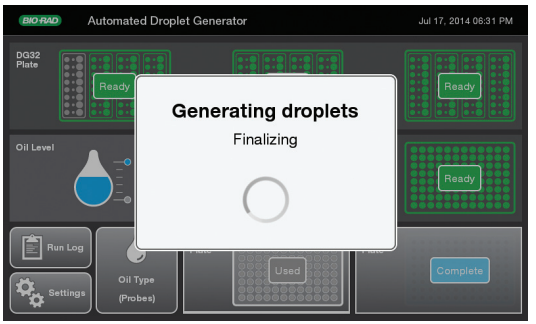


Fig. 18. A, touching the Abort button will bring up a confirmation screen; B, system display with time since run aborted.

14. Once the plate of droplets is ready, the screen will display a finalizing window followed by a blue **Droplets ready** message with a timer showing time elapsed since complete.

A



B

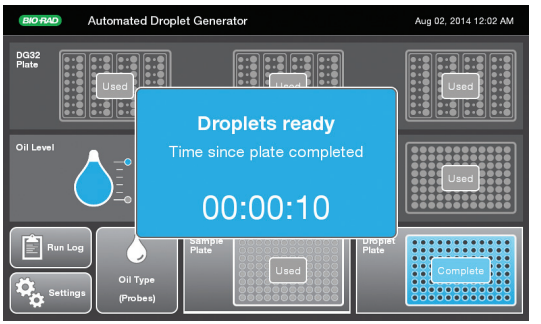


Fig. 19. A, finalizing display at the end of a run; B, count-up display indicating the time that has elapsed since droplet generation completed.

The Droplet Plate at the front right corner of the instrument will be illuminated blue. The corresponding icon on the touch screen will also pulse blue. The door on the instrument will unlock and the droplet plate can be removed. Please wait to remove the oil bottle as noted on the screen; the unused oil is being returned to the bottle.

If any errors were encountered during the run, an error notification will appear below the count-up timer. Touch the **Errors encountered** button to bring up the run logs and identify the error.

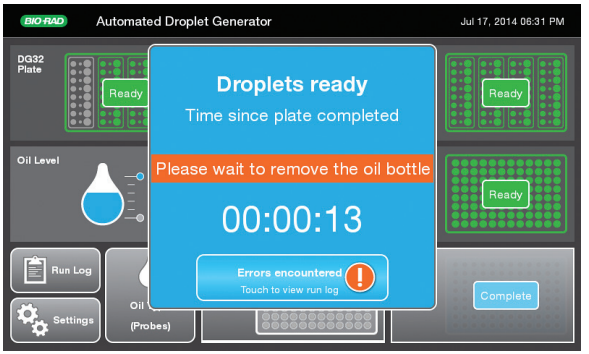


Fig. 20. Droplet generation is complete. Please wait to remove the oil bottle until the orange message disappears from the screen.

15. Remove the droplet plate containing ddPCR droplets and seal within 30 min of droplet generation completing. Use the PX1 PCR Plate Sealer and foil seals listed in Table 2. Follow the instructions in the PX1 PCR Plate Sealer Instruction Manual (part number 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 red stripe on the foil seal should face up towards the user.) 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 will open automatically. Remove the plate from the block for thermal cycling. Remove the block from the PX1 Sealer.
    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.
16. Remove any consumables from the AutoDG instrument that have been completely used and discard. DG32 cartridges, tips and plates are single-use consumables and are not reusable. The AutoDG instrument will remember the status of the consumables as long as it remains powered on; if a consumable has been only partially used, leave it in the AutoDG instrument for the next run.

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.

Fig. 21. PX1 PCR Plate Sealer (left) and a sealed 96-well plate (right).

2.3 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, IVD (follow the instructions in the QX200 Droplet Reader, IVD Instruction Manual, part number 10000044967).

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, IVD Instruction Manual (part number 10000044967) for more details.

2.4 Advanced Loading and Switching of Automated Droplet Generation Oils

When a new or different bottle of oil is loaded, or when power is lost, the Automated Droplet Generator, IVD will perform a small volume flush and prime of the oil delivery system. If the AutoDG instrument has not been used for an extended time period (a week or more) but left powered on, it is recommended to perform a flush and prime routine before running a plate.

2.4.1 Loading a Bottle of Oil before Configuring a Plate

To save time at the beginning of a run, a bottle of Automated Droplet Generation Oil can be loaded into the instrument, and the oil delivery system primed, at any point. When there is no bottle of oil loaded into the instrument, the Oil Level display on the screen will appear gray to indicate it is empty. The Oil Type display on the screen will not display an oil type.

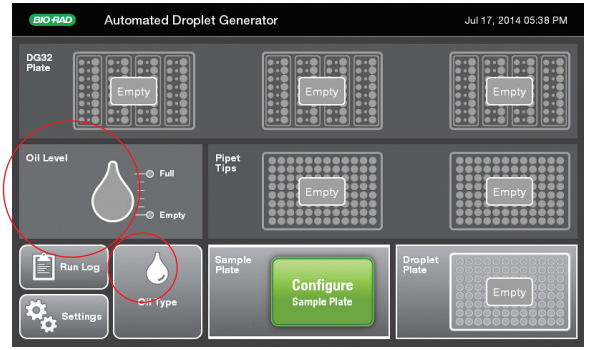


Fig. 22. Home screen with oil level and oil type waiting to be set.

1. Open the door of the AutoDG instrument by lifting up on the handle until it clicks into a locked position.
2. Remove the cap from the bottle of AutoDG Oil and insert the bottle into the tower of the oil delivery system at the front left corner of the instrument.
3. Touch the **Oil Type** button on the home screen to bring up the Select Oil Type display.
4. Touch the droplet to indicate which type of oil has been loaded. To help differentiate, the AutoDG Oil for Probes bottle has a yellow label and contains a yellow straw, while the AutoDG Oil for EvaGreen bottle has a green label and contains a green straw.

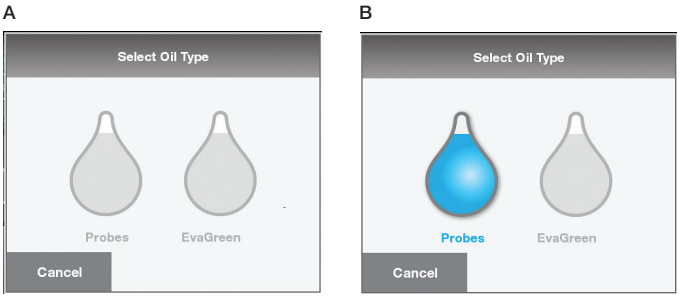


Fig. 23. A, oil selection display; B, AutoDG Oil for Probes selected.

5. The instrument will check that there is oil in the bottle. If you did not load a bottle (or insert the bottle completely) as described in step 2, you will be prompted to load a bottle of the oil type that you selected in the previous screen. Once correctly loaded, the AutoDG instrument will begin the flush and prime routine.

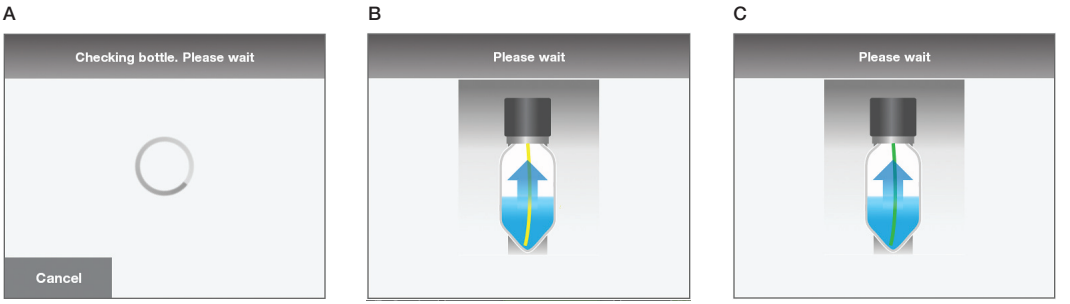


Fig. 24. A, display while checking for the bottle; B, display while priming the oil delivery system with AutoDG Oil for Probes; C, display while priming the oil delivery system with AutoDG Oil for EvaGreen.

6. Once complete, the instrument will display a screen confirming successful priming of the selected and loaded oil.

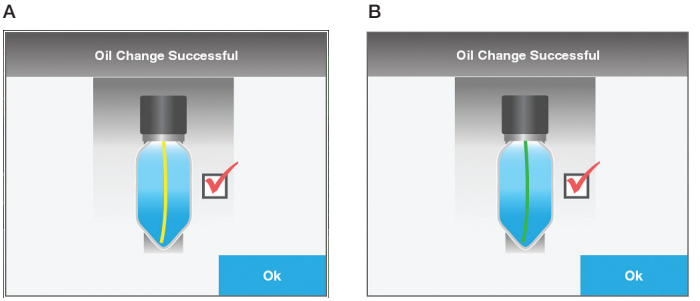
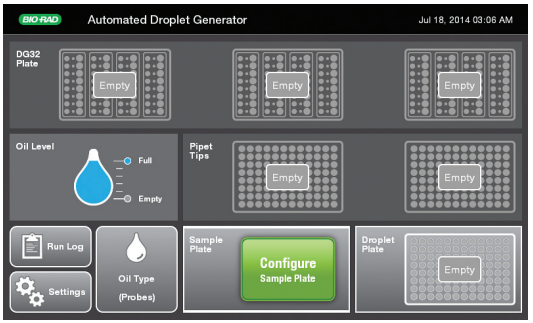


Fig. 25. A, successful priming of AutoDG Oil for Probes; B, successful priming of AutoDG Oil for EvaGreen.

7. Touch **OK** to return to the home screen. The Oil Level icon will now reflect the amount of oil in the bottle, and the Oil Type icon will reflect the type of oil loaded and primed.

A



B

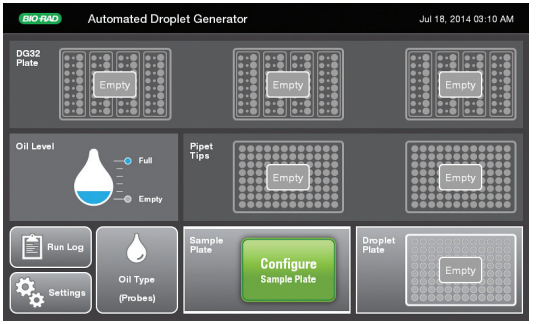


Fig. 26. A, home screen displaying a full bottle of AutoDG Oil for Probes loaded; B, a partial bottle of AutoDG Oil for Probes loaded.

2.4.2 Changing between AutoDG Oil Types

Changing between Automated Droplet Generation Oils can be performed at any time, and the oil delivery system primed. The flush and prime when changing chemistries is important for good ddPCR results.

The AutoDG instrument will perform the flush and prime at the start of any run where the oil selected differs from the previous run. Changing oils ahead of time saves time at the beginning of the droplet generation process. Because the AutoDG instrument keeps track of the type of oil used in a previous run, so long as it is powered on, it will perform this operation before beginning a plate of droplets with a different type of oil.

To perform the oil change at any time:

1. Open the door of the AutoDG instrument by lifting up on the handle until it clicks into a locked position.
2. Remove the bottle currently loaded and replace the cap tightly for storage.
3. Remove the cap from the alternate type of AutoDG Oil and insert the bottle into the tower of the oil delivery system at the front left corner of the instrument.
4. Touch the **Oil Type** button on the home screen to bring up the Select Oil Type display.
5. The oil type previously set in the instrument will be selected in blue. Touch the other type of oil to activate the **Change Oil** button.

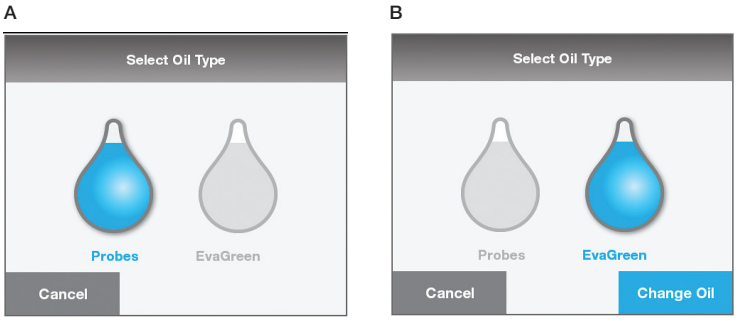


Fig. 27. An example of switching oil types. A, oil selection display with Probes selected; **B**, **Change Oil** button activated by touching the EvaGreen droplet.

6. The instrument will check that a new bottle has been properly loaded and that there is oil in the bottle. If you did not load a bottle (or insert the bottle completely) as described in step 3, you will be prompted to load a bottle of the oil type that you selected in the previous screen. Once correctly loaded, the AutoDG instrument will begin the flush and prime routine.
7. Once complete, the instrument will display a screen confirming successful priming of the selected and loaded oil.
8. Touch **OK** to return to the home screen. The Oil Level icon will now reflect the amount of oil in the bottle, and the Oil Type icon will reflect the type of oil loaded and primed, which will now be different than previously displayed.

2.5 Accessing Run Logs

The AutoDG instrument stores information about each run, including consumables used, rows of reactions processed, and any errors that may have occurred.

To access the run log:

1. Touch the **Run Log** button on the home screen.
2. To display detailed information about a run, touch the run.
3. Touch **OK** to return to the run log (and to export run log or exit).

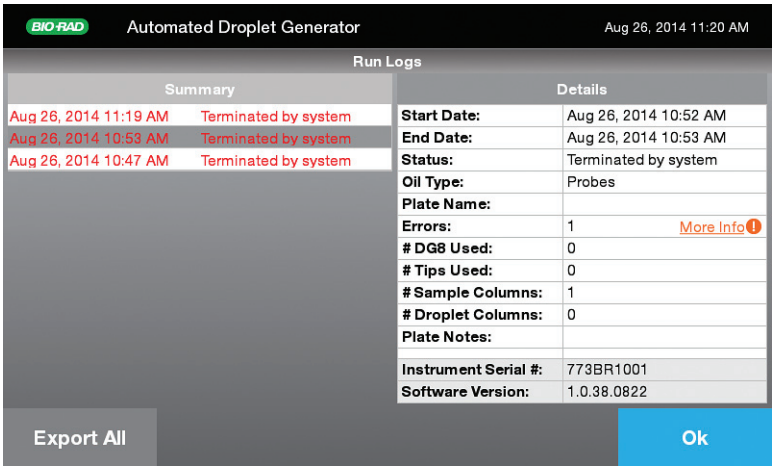


Fig. 28. AutoDG instrument run log displaying runs completed or terminated.

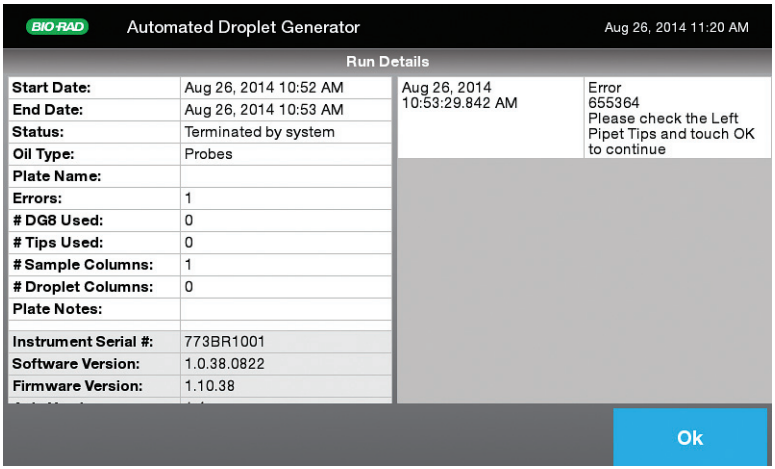


Fig. 29. Example of Run Details for a run terminated by the system due to a pipet tip box lid being left on when placed in the instrument. The run details are displayed on the left of the screen and the error details on the right.

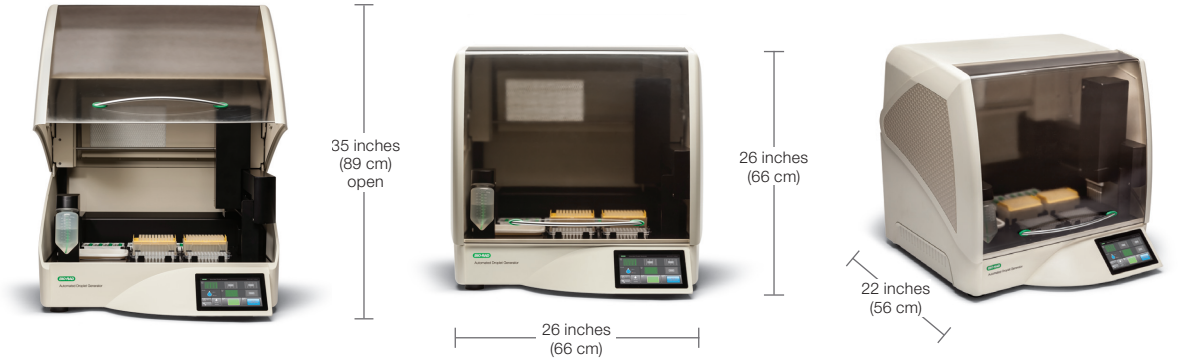
To export the run log:

1. Touch the **Run Log** button on the home screen.
2. Remove the AutoDG instrument's side panel to the right of the touch screen. Slide the magnetic panel down to release, then pull straight out to remove the panel.
3. Insert a USB key into the USB port on the side of the instrument.
4. Touch the **Export All** button at the bottom left of the screen.
5. Wait while the system exports the run logs to the USB key.
6. When finished, **Run logs successfully exported** will be displayed on the screen. It is now safe to remove the USB key.
7. Replace the side panel.

If the USB key is not connected, the AutoDG instrument will display **USB key not found. Please insert it and try again.** Insert the USB key and touch **Retry** to proceed.

3 Specifications and Maintenance

3.1 Specifications



Weight	100 lb (45.4 kg)
Size (W x D x H)	26 x 22 x 26" (66 x 56 x 66 cm)
Electrical requirements	100–240 V, 50/60 Hz, 90 W; voltage fluctuations not to exceed +10% of ratings (for external power supply provided)
Fuse	4 A, 24 V internal (not user serviceable)
Temperature	18–30°C, optimal range is 23 ± 2°C
Altitude	0–6,500 ft (0–2,000 m), optimal range is 0–500 m
Humidity	50% max (noncondensing)
Pollution degree	2 (indoor use)
Installation category	II (external power supply plugs into standard AC receptacle)
Ventilation requirement	5" (13 cm) left and right of machine and 2" (5 cm) behind should be unobstructed for proper ventilation

3.2 Maintenance

3.2.1 General Care

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 on all surfaces except the door. 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.

Do not replace detachable power cord with an uncertified or an inadequately rated cord.

The Automated Droplet Generator, IVD is verified to be functioning to manufacturer specifications upon installation.Yearly preventative maintenance offered by Bio-Rad is recommended to ensure the instrument continues to function according to specifications.

In the event of malfunction and/or changes in the analytical performances of the device. Please contact Bio-Rad Technical Support (1-800-424-6723).

3.2.2 Software and Firmware Updates

If Bio-Rad informs you that a new version of software and firmware are available for the Automated Droplet Generator, IVD, the upgrade can be performed with a USB key.

1. Remove AutoDG instrument's side panel to the right of the touch screen. Slide the magnetic panel down to release, then pull straight out to remove the panel.
2. Insert a USB key into the USB port on the side of the instrument.
3. Touch the **Settings** button on the home screen.
4. Scroll down to the Update section of the menu and touch **Update Software and Firmware**.
5. Touch the **Update** button and wait while the system completes the upgrade.
6. When finished, a message will appear indicating it is safe to remove the USB key.
7. Replace the side panel and power cycle the instrument.

3.2.3 Cleaning the Oil Purge Reservoir

It is recommended that you clean the oil purge reservoir on a quarterly basis, or more frequently if switching Droplet Generation Oil chemistries (probe/EvaGreen) frequently. The oil purge reservoir collects Droplet Generation Oil during the flush and prime routine; it is unused oil that has not come into contact with the ddPCR™ reaction. To access the oil purge reservoir:

1. Touch the **Settings** button on the home screen.
2. Under the System Maintenance section of the menu, touch **Remove Oil Purge Reservoir**.

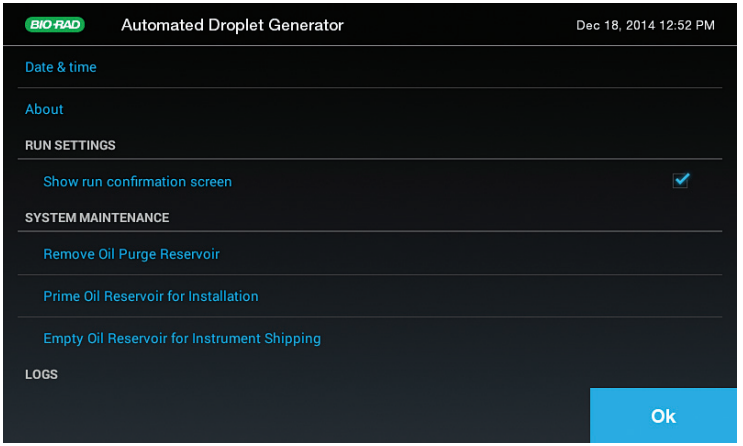


Fig. 30. System Maintenance section of the Settings menu.

3. If the door is open it will close automatically and a message will appear. Touch **OK** to continue.
4. The droplet generation head of the AutoDG instrument will slide out of the way, exposing the small oil purge reservoir. The screen will display a **Moving droplet generation head** message while this occurs.
5. Once complete, the onscreen instructions will prompt you to remove the oil purge reservoir, clean, and replace it. Open the door and remove the reservoir from the back left corner of the instrument deck. The reservoir is magnetic and can be lifted easily.
6. Wash the oil purge reservoir with distilled water, dry completely, and replace.
7. Touch the **OK** button to close the door and move the droplet generation head back into place. The **Moving droplet generation head** message will once again be displayed while this occurs.
8. When finished, a complete message will appear, and you can touch **OK** to return to the Settings menu.

4 Automated Droplet Generator, IVD Troubleshooting

The Automated Droplet Generator, IVD provides onscreen troubleshooting should an error occur. Guidance will be provided as to how to best resolve the error, or the instrument will advise that Bio-Rad Technical Support be contacted.

4.1 Restarting a Run after an Error

If the AutoDG™ Instrument encounters an obstruction during the run, it will stop the run and display an error message with the area of the deck that needs attention.

1. Open the door and remove the obstruction and/or replace the consumable indicated in the onscreen error message.
2. Close the door and touch **OK** on the error display.
3. Touch the **Configure Sample Plate** icon on the home screen; the AutoDG instrument remembers the status of all consumables so long as it remains connected to a power supply.
4. On the screen, select the columns of samples remaining to be processed and touch **OK**.
5. Touch the **Start Droplet Generation** icon to resume the run.

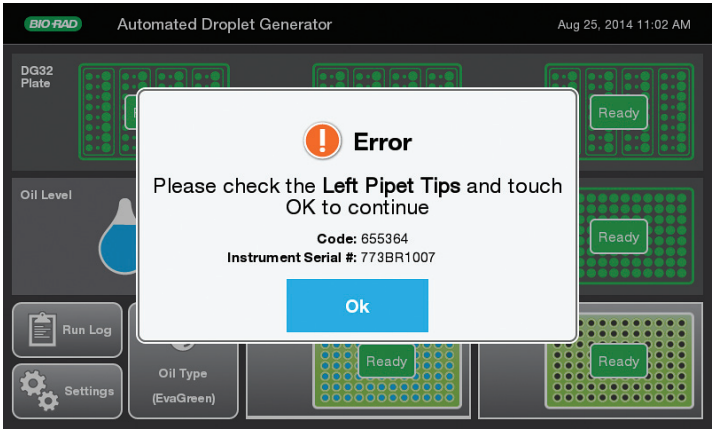


Fig. 31. Example of error message displayed when the lid is left on the box of tips placed in the left tip box holder. The run stops and an error message indicating the position of the error displays on the touch screen.

4.2 Restarting a Run after a System Error

If the AutoDG instrument encounters a system error during the run, it will stop the run and display an error message requiring power cycling.

1. Unplug the AutoDG instrument from the power supply.
2. Wait 30 sec and plug it back in.

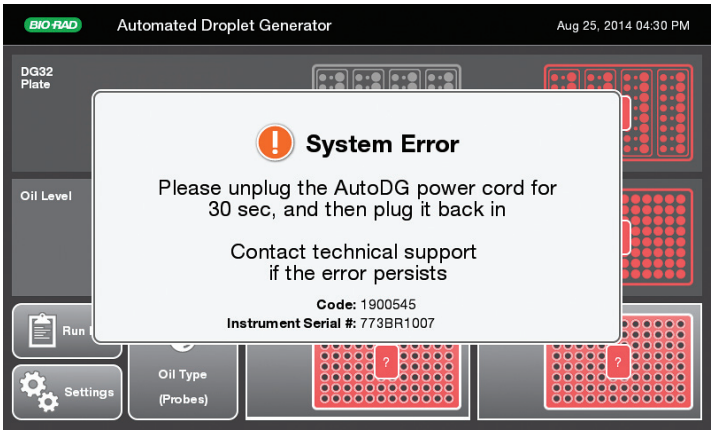


Fig. 32. Example of System Error message. The run stops and power cycling the instrument is required.

3. The AutoDG instrument will not be able to recall the status of all consumables and will require manual confirmation (see Section 2.1).
4. Reset the unused consumables and touch the **Configure Sample Plate** icon on the home screen.
5. Select the columns of samples remaining to be processed on the screen and touch **OK**.
6. Touch the **Start Droplet Generation** icon to restart the run.

4.3 Exporting Logs for Technical Support

In the event of a system error that cannot be resolved, please contact Bio-Rad Technical Support as indicated on the touch screen. The error number and serial number of your AutoDG instrument are provided on the screen; please provide these to the technical support representative.

You will also be asked to export and submit the history and run logs for the Automated Droplet Generator, IVD. Please note that no sample data is transferred in this process, just the run log of the instrument. To export both history and run logs together:

1. Remove the side panel of the AutoDG instrument to the right of the touch screen. Slide the magnetic panel down to release, then pull straight out to remove the panel.
2. Insert a USB key into the USB port on the side of the instrument.
3. Touch the **Settings** button on the home screen.
4. Scroll down to the **Logs** section of the menu and touch **Export all logs**.
5. Please wait while the system exports the instrument logs to the USB key.
6. When finished, an export complete message will appear indicating it is safe to remove the USB key.
7. Replace the side panel.

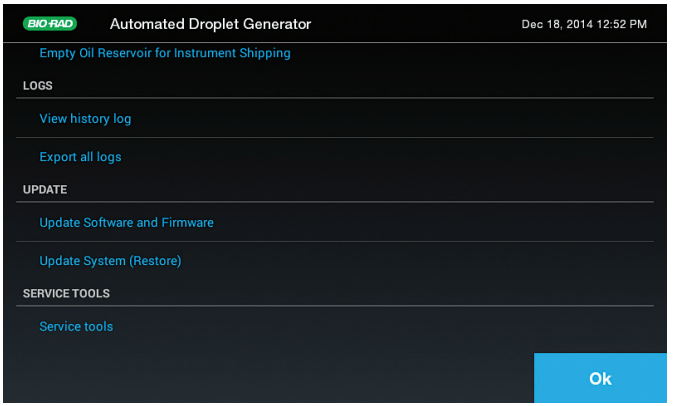


Fig. 33. Logs section of the Settings menu.

4.4 Additional Troubleshooting

Use the following table to help you troubleshoot issues with your AutoDG instrument. If you have additional questions or if the recommended action does not resolve the problem, please contact Bio-Rad Technical Support.

Table 4. Additional troubleshooting for the AutoDG instrument.

Issue	Possible Causes	Action
No screen display No response when you power on the instrument	Not connected to power source	Check power cord is securely connected
Screen display is missing characters, illegible, or not responsive to touch	LCD screen failure	Request service
Door won't open or is hard to open	Instrument is running Instrument is disconnected from a power supply	Wait for droplet generation to complete or touch the **Abort** button on the screen to terminate the run early. Once movement has stopped inside the instrument, the door will unlock and can be opened easily. Connect the instrument to a power supply and wait for initialization to complete; the door will unlock and can be opened easily.
Light under a consumable stays yellow	Consumable is not inserted all of the way or is in the incorrect orientation	Try positioning the consumable in a different orientation
Red Oil Level icon	AutoDG Oil bottle contains less than a full plate's worth of oil and will require additional oil to complete the next run	Be prepared to insert a new bottle of oil during the setup process for the next plate

Ordering Information

QX200™ AutoDG™ ddPCR™ System, IVD

Catalog #	Description
17002229	**QX200 AutoDG ddPCR System, IVD**, includes Automated Droplet Generator, IVD, QX200 Droplet Reader, IVD, laptop computer, QuantaSoft Software
12001630	**Automated Droplet Generator, IVD**, includes Automated Droplet Generator, IVD, power cord, and accessories
12001045	**QX200 Droplet Reader, IVD**, includes droplet reader, ddPCR manual, plate holders, USB cable, power cord
12001922	**ddPCR Dx AutoDG Consumable Pack,** includes 3 x 5 ddPCR 96-well plates, 1 x 50 ddPCR Pierceable Foil Heat Seals, 1 x 50 ml Automated Droplet Generation Oil for Probes, 1 x 15 DG32 Cartridges, 10 x 96 Pipet Tips, and instructions for use required for automated droplet generation
12002526	**ddPCR Dx Droplet Reader Oil Pack,** includes 1 x 1,000 ml Droplet Reader Oil required for Droplet Digital PCR

ddPCR Reagents

Catalog #	Description
12003031	**ddPCR Dx AutoDG Supermix Pack,** includes 5 x 1 ml supermix required for Droplet Digital PCR
186-3052	**ddPCR Buffer Control for Probes**, 9 ml (2 x 4.5 ml), 2x buffer
186-4052	**QX200 Buffer Control for EvaGreen**, 9 ml (2 x 4.5 ml), 2x buffer

Thermal Cyclers and Plate Sealer

Catalog #	Description
185-1196	**C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module**, includes C1000 Touch Thermal Cycler chassis, 96-well reaction module, USB flash drive
184-1000-IVD	**C1000 Touch Thermal Cycler with 96–Deep Well Real-Time PCR Reaction Module**, includes C1000 Touch Thermal Cycler chassis, 96–deep well 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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Czech Republic *420 241 430 532* ***Denmark*** *45 44 52 10 00* ***Finland*** *358 09 804 22 00*
France *33 01 47 95 69 65* ***Germany*** *49 89 31 884 0* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100*
India *91 124 4029300* ***Israel*** *972 03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460*
Mexico *52 555 488 7670* ***The Netherlands*** *31 (0)318 540 666* ***New Zealand*** *64 9 415 2280*
Norway *47 23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 (0) 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *46 08 555 12700*
Switzerland *41 026 674 55 05* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311*
United Arab Emirates *971 4 8187300* ***United Kingdom*** *44 020 8328 2000*

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