ddPCR™ Dx Universal Kit for QX200™ AutoDG™ ddPCR™ Dx System

Instructions For Use

REF

17001378 - ddPCR™ Dx Universal Kit for QX200™ AutoDG™ ddPCR™ Dx System

Σ 480

CE

IVD

Revision: B

12005433revB
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Translations:

Product documents may be provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
 Number of Tests	USE For use with	SN Serial Number
Rx Only Prescription Use Only	UDI-DI Unique Device Identification-Device Identifier	 Contains Latex

Bio-Rad Technical Support

For help and technical advice, please contact the Bio-Rad Technical Support department. In the United States, the Technical Support department is open Monday–Friday, 5:00 AM–5:00 PM, Pacific time.

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Online technical support and worldwide contact information are available at www.consult.bio-rad.com.

Legal Notices

Safety and Regulatory Compliance

QX200™ AutoDG™ System has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

1. IEC 61010-1:2010 (3rd ed.), EN61010-1:2010 (3rd ed). Electrical Equipment for Measurement, Control, and Laboratory Use — Part 1: General requirements

2. EN 61326-1:2006 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements

3. UL 61010-1:2004, Laboratory equipment, Test & Measurement Equipment and Industrial Process Controls

4. 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 their own expense.

CE The CE mark indicates that the manufacturer ensures the product conforms with the essential requirements of the European Directive for in-vitro diagnostic medical devices 98/79/EC.

The CSA mark indicates that a product has been tested to Canadian and U.S. standards, and it meets the requirements of those applicable standards.

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

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.

ddPCR™ Dx Universal Kit for QX200™ AutoDG™ ddPCR™ Dx System, Warnings and Precautions

For in vitro diagnostic use. For healthcare professional use.

This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.

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:

5. Butyl gloves are made of a synthetic rubber and protect against peroxide, hydrofluoric acid, strong bases, alcohols, aldehydes, and ketones

6. Natural (latex) rubber gloves are comfortable to wear and feature outstanding tensile strength, elasticity, and temperature resistance

7. 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

8. 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

Table of Contents

In Vitro Diagnostic Medical Device.

Common Name: **Dx Universal Kit for QX200™ AutoDG™**

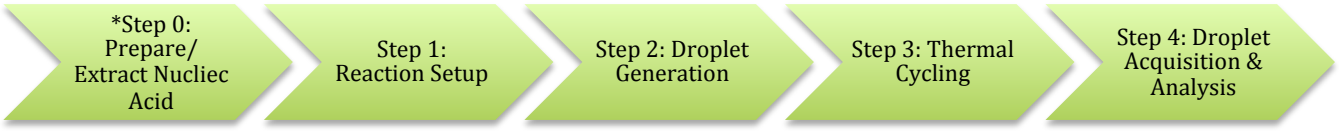
Name: **ddPCR™ Dx Universal Kit for QX200™ AutoDG™ ddPCR™ Dx System**
p/n: 17001378

Intended Use:

The ddPCR™ Dx Universal Kit for QX200™ AutoDG™ ddPCR™ Dx System (p/n: 17001378) is a set of non-analyte-specific reagents and consumables required to be used on QX200™ Droplet Digital PCR System in order to generate and read droplets. The end user supplies the diagnostic assay, controls and calibrators.

Principle of Procedure:

ddPCR™ DX Universal Kit for QX200™ AutoDG™ ddPCR™ Dx System, is a kit comprising of reagents and consumable needed to run a ddPCR™ reaction workflow on the QX200™ system, except primers, probe(s), and template.



*Step 0 is needed should there be a need to convert RNA into cDNA.

The Dx Universal Kit is designed and packaged to simplify and enable the use of QX200™ ddPCR™ System in open mode. Allowing the laboratory to develop an assay of interest without the need to figure out common consumables necessary for the instrument and the technology. Thus enabling the lab to focus more on designing their primers, probe(s) and template sequences.

The kit consists of a ddPCR™ Dx AutoDG™ Consumable Pack, ddPCR™ Dx AutoDG™ Supermix Pack, and ddPCR™ Dx Droplet Reader Oil Pack.

Reagents & Supplies:

Materials Provided

Description:	Σ	Catalog #
ddPCR™ Dx Universal Kit for QX200™ AutoDg™ ddPCR™ Dx System	**480**	**17001378**

NOTE: Catalog # below (120001922, 12003031 and 12002526) all are included in ddPCR™ Dx Universal Kit for QX200™ System

ddPCR™ Dx AutoDG™ Consumable Pack **480 rxns** **12001922**

Component Number	Component	Quantity	Description/Presentation
12003185	ddPCR™ 96-Well Plates	3 x 5	15 Ready for Use 96-well plates
12003015	ddPCR™ Pierceable Foil Heat Seal	1 x 50	1 bag of 50 heat sales Ready for use
12003017	Droplet Generation Oil for Probes	1 x 50 mL	50 mL bottles for oil Ready for use
12003016	DG32™ Cartridges	1 x 15	1-15 count package of droplet generation cartridges Ready for use
12003010	AutoDG™ Pipette Tips	1 x 10	10 packages of pipette tips for use with AutoDG™
16001685	Instruction Manual	1	Instructions for use with AutoDG™

ddPCR™ Dx AutoDG™ Supermix Pack **480 rxns** **12003031**

- 5 x 1mL thaw to room temperature before using Supermix for QX200™ AutoDG™ System

ddPCR™ Dx Droplet Reader Oil Pack **784 rxns** **12002526**

- 1 x 1L ready to use Droplet Reader Oil for use with QX200™ AutoDG™ System

Materials required but not provided:

Description:	Catalog #
• QX200™ AutoDG™ Dx Digital Droplet PCR System	17002229
○ QX200™ Droplet Reader, IVD	12001045
▪ QuantaSoft Software	10026368
▪ Plate Holders (2)	29711024
▪ USB 2.0 cable	Call technical support

- Power Cord — Call technical support

- QX200™ Automated Droplet Generator, IVD — 12001630
  - Cartridge Holder — 186-3051
  - Power Cord — Call technical support
  - Cooling Block Accessory — Call technical support
  - Oil Purge reservoir — Call technical support

- Pipets
  - 20 **µL** pipet for sample loading — Rainin L-20
  - 50 **µL** pipet for droplet transfer — Rainin L8-50
  - 8-channel, 200 **µL** pipet for oil — Rainin L8-200

- Pipet Tip (filtered) — Rainin GP-L10F, GP-L200F

- C100 Touch™ Thermal Cycler with 96-deep well block, IVD — 184-1000-IVD

- PX1™ PCR Plate Sealer — 181-4000

Storage and Stability:

ddPCR™ Dx AutoDG™ Supermix is stable at -25°C to -15°C through the stated expiration date printed on the labels. Once thawed, the supermix can be stored at 4°C for up to 2 weeks. Repeated freezing and thawing of the supermix is not recommended.

ddPCR™ Dx AutoDG™Consumable Pack is stable at 15-30°C (room temperature) through the stated expiration date printed on the labels.

ddPCR™ Dx Droplet Reader Oil Pack is stable at a constant temperature of 15-30°C (room temperature) through the stated expiration date printed on the labels.

Warnings and Precautions:

- This product is For Laboratory Use
- Do not use expired materials
- Always handle specimens in accordance with safe laboratory procedures such CLSI Document M29[1]
- Protect reagents against heat and humidity
- Wear personal protective equipment, while handling all reagents. Wash hands thoroughly after performing the test.
- Dispose

Quality Control:

ddPCR™ Dx AutoDG™ Supermix for Probes (No dUTP) is free of contaminating DNase and RNase. Stringent specifications are maintained to ensure lot-to-lot consistency.

Specimen Collection:

- Collect specimens and label appropriately
- Collected specimens should be maintained per the conditions validated by the user
- For best practices, collect whole blood samples according to standard techniques at each institution. Collect a minimum of 5 mL blood specimens in tubes containing EDTA as anticoagulant.
- Proceed to sample and reaction preparation

Sample & Reaction Preparation:

Sample Extraction is required. Input to ddPCR workflow is extracted DNA or cDNA.
Application specific extraction preparation procedures should be developed and validated by the user.

- Common sample preparation methodologies for extraction of DNA from whole blood or buffy coat are compatible with ddPCR™ methods.
- Common sample preparation methodologies for extraction of DNA from FFPE tissue are compatible with ddPCR™ methods.
- When working with RNA common sample preparation methodologies and Reverse Transcription PCR should be deployed at each institution.

Recommendation for Optimal Results:

- Follow general guidelines and recommendations for ddPCR™ Dx Automated Droplet Generator, IVD instruction manual.
- The concentration of human genomic DNA should be 30 pg – 330 ng per 20 µL reaction digested..

DNA Digestion:

DNA fragmentation by restriction digestion prior to droplet generation enables optimal accuracy by separating tandem gene copies, reducing sample viscosity, and improving template accessibility for input samples. Restriction digestion improves overall performance of digital PCR across applications.

Recommendation: choose a restriction endonuclease that does not cut either the target or reference amplicon and that is insensitive to methylation. Four-base cutters and high-fidelity enzymes are preferred.
Two Strategies may be used to perform restriction digestion of DNA samples: digestion directly in the ddPCR reaction during setup, or conventional digestion prior to ddPCR.

DNA Digestion in ddPCR™ Reaction:

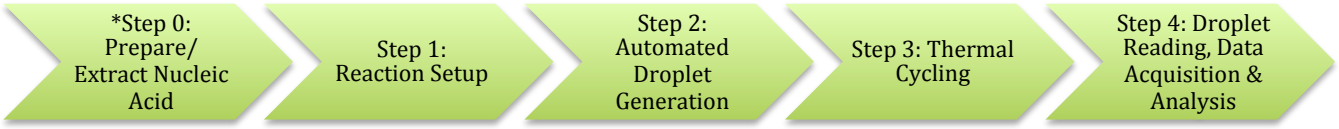
- Efficient digestion of sample DNA can be achieved by direct addition of restriction enzymes to the ddPCR reaction.
- Dilute the restriction enzyme using the recommended diluent buffer according to the manufacturer's instructions, and 1 µL to the ddPCR™ reaction.
- Approximately 2-5 units of restriction enzyme per 25 µL ddPCR™ reaction are recommended.
- The addition of restriction enzyme buffer with high salt can inhibit digital PCR and should be avoided.
- HaeIII, MseI, AluI, HindIIII, and CviQI have been observed to work well for digestions in ddPCR™ reactions.

DNA Digestion Prior to ddPCR™ Reaction:

- Restriction Enzyme digestion can be carried out as a separate reaction before ddPCR™ reaction setup.
- Use 5-10 enzyme units per microgram DNA, and 10-20 enzyme units per microgram genomic DNA.
- Incubate the reaction for 1hr at the temperature recommended for the restriction enzyme.
- Heat inactivation is not required, but can be considered if long-term storage is required; do not heat inactivate above 65°C.
- DNA purification is not necessary after restriction digestion.
- Use a minimum of 10-fold dilution of the digest to reduce the salt content of the sample in ddPCR reaction.
- Store digested DNA at -20°C or below.
- Prepare the ddPCR™ reaction mix

Procedure:

Workflow:



*Step 0 is needed should there be a need to convert RNA into cDNA.

Reaction Preparation & Setup:

1. Thaw all supermix, primers and probes components to room temperature. Mix thoroughly by vortexing the tube to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of the tube.
2. Prepare samples at the desired concentration before setting up the reaction mix.
3. Prepare the reaction mix for the number of reactions needed.
4. Assemble all required components except the sample, dispense equal aliquots into each reaction tube, and add the sample to each reaction tube as the final step.
5. Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube. Allow reaction tubes to equilibrate at room temperature for about 3 minutes.

Table 1:

Component	Volume per Reaction (µL)	Final Concentration
ddPCR Supermix for Probes (No dUTP)	12.5	1x
20x target primers/probe (FAM)	1.25	900 nM/250 nM
20x reference primers/probe (HEX/VIC)	1.25	900 nM/250 nM
Diluted restriction enzyme (see DNA Digestion)	1.00	Variable
Sample	Variable	Up to 330 ng*
RNase & DNase Free Water	Variable	-
Total Volume	25	-

* Sample concentrations >66ng per reaction and certain applications may require restriction digestion for optimal target detection. If digestion is not required, prepare the ddPCR reaction mix without the diluted restriction enzyme.

Upon completion the reaction preparation & setup, move forward to step 2 – Droplet Generation workflow.

Automated Droplet Generation:

Refer to the QX200™ AutoDG™, IVD System Instruction Manual (REF 10000067122) for a detailed graphic driven setup & use instructions. ,

Note: all 8 wells in a single column in the DG32 Droplet generator cartridge must contain sample or no template control.

Automated Droplet Generator, IVD prepares droplets for up to 96 samples at a time. Droplet generation takes ~45 minutes for 96 well plate.

Upon completion of droplet generation, from Automated Droplet Generator, pick up the 96-well plate from the front of the AutoDG™ system and seal the 96-well plate containing the droplets with a PX1 PCR Plate Sealer.

Follow the instructiosn in the PX1 PCR Plate Sealer:

- Set the plate sealer temperature to 180°C and time to 5 sec (not default conditions).
- 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.
- 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.) Red stripe should be visible when the foil is place on the plate. Do not attempt to place the frame over the foil-covered plate. The frame is only for use with other seals.
- 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.
- 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.
- 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.

Thermal Cycling:

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

Table 2. Cycling conditions for Bio-Rad's C1000 Touch Thermal Cycler*

Cycling Step	Temperature (°C)	Time	Ramp Rate	Number of Cycles
Enzyme Activation	95	10 min	2°C/sec	1
Denaturation	94	30 sec		40
Annealing/Extension	60	1 min		40
Enzyme Deactivation	98	10 min		1
Hold (optional)	4	Infinite		1

* Use a heated lid set to 105°C and set the sample volume to 40 µL.
* Modify thermal cycling conditions as required for individual assay.

Warning: using a different ramp rate may impact the pcr-amplification and results.

Data Acquisition and Analysis:

1. After thermal cycling, place the sealed 96-well plate in QX200™ Dx Droplet Reader
2. Open QX200™ software (QuantaSoft™) to set up a new plate layout according to your experimental design.
3. Refer to the QX200™ Dx Droplet Reader Manual (REF 10000044967) for detailed instructions.

4. Designate the following information in the software:
   - Sample Name
   - Experiment type
   - Supermix Type: ddPCR Supermix for Probes (No duTP)
   - Target Names
   - Target Type(s): (Ch1 for FAM and Ch2 for HEX/VIC)

5. Select "Apply" to setup the plate layout, and select "OK" to finish.
6. Upon completion, select "RUN" to begin the droplet reading process (Data Acquisition).
7. After Data Acquisition is complete, analyze and review output.
8. The output reported is copies/ µL of the final 1x ddPCR™ reaction.

Notes:

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