
Instructions for Use for the RDG16 Cartridge, Above 500m Altitude

Version 1.0

| Catalog Number | Product Reference Number | Product Description |
|----------------|--------------------------|------------------------------|
| 12025252 | C16011 | RDG16 Cartridges, Pack of 12 |

November 2025

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Purpose and Intended Use

The RDG16 Cartridge is a consumable used with the QX700 Droplet Digital PCR System, the Nio Digital PCR system, and the naica system to perform Droplet Digital PCR (ddPCR™). It partitions nucleic acid samples into water-in-oil droplets and contains them on the cartridge for amplification and nucleic acid detection. The cartridge is intended for use by laboratory personnel trained in ddPCR with the QX700 ddPCR System, the Nio Digital PCR system, or the naica system.

This document provides information and instructions for using the RDG16 Cartridge when operating the

compatible systems at altitudes above 500 meters.

Important: Contact Bio-Rad Technical Support for the correct protocols to run on the instruments.

Note: To use this product at lower altitudes, refer to the Instructions for Use for the RDG16 Cartridge.

About the RDG16 Cartridges

The RDG16 Cartridge is a ready-to-use, oil-filled consumable designed for ddPCR.

- The cartridge contains 16 wells, arranged in two columns of 8 wells.
 - Left column: A1–H1 (light grey)
 - Right column: A2–H2 (dark grey)
- Pipette individual sample mixes into each cartridge well through the inlet port by piercing the aluminum foil cover.

Note: Bio-Rad recommends piercing the foil cover with the piercing tool before pipetting the PCR mix.

Important: Keep the RDG16 cartridges in the plate during the pipetting step.

- Central side handles allow for easy handling and transport.
- Each cartridge is marked with a unique serial number (cartridge ID), engraved on the cartridge and encoded in a scannable barcode.
- The expiration date is engraved on the cartridge.

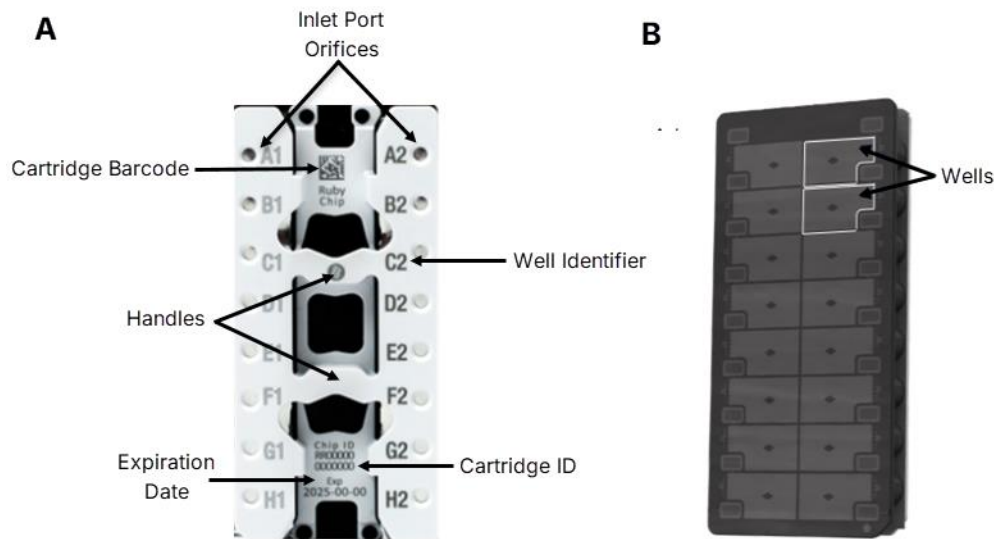


Figure 1: Top view of the RDG16 Cartridge (left) and bottom view of the RDG16 Cartridge (right)

Table 1: General characteristics

| Item | Specification |
|------------------------|---|
| Capacity | 16 samples/cartridge |
| Input volume | 5 µ/well |
| Droplets per sample | 10,000 – 17,000** |
| Average droplet volume | ~ 0.22** nL |
| Dynamic range* | ~ 4.7 log |
| LOD min (95%)* | down to 0.80**cp/µL |
| LOD max (95%)* | up to 39,000**cp/µL |
| Altitude | Operating at max. 1000 m above sea level. Note: The information in this IFU applies to an altitude above 500 m only |













(*) Analytical specifications are assay dependent/Values presented here are theoretical and are calculated based on the droplet size, droplet volume and numbers.

(**) Results obtained with naica multiplex PCR MIX and naica PCR MIX.

Table 1: System compatibility

| Item | System | Part Number |
|----------------------------------|-------------------------------------|---------------|
| QX700 ddPCR System compatibility | QX700 HT Droplet Digital PCR System | 17011036 |
| | QX700 S Droplet Digital PCR System | 17010638 |
| | QX700 E Droplet Digital PCR System | 17010628 |
| Nio Digital PCR system | Nio E | N30027.2 |
| | Nio | N30027.1 |
| | Nio+ | N30027.0 |
| naica system compatibility | Geode | H13000-H15000 |
| | Prism3 | H22000 |
| | Prism6 | H24000.6 |

Symbols Lexicon

| | |
|---|--|
|  <p>Manufacturer</p> |  <p>Distributor</p> |
|  <p>Catalog Number</p> |  <p>Batch Code</p> |
|  <p>Use By Date</p> |  <p>Temperature Limit</p> |
|  <p>Do Not Use if Package is Damaged and Consult Instructions for Use</p> |  <p>Consult Instructions for Use</p> |
|  <p>Keep Dry</p> |  <p>Do Not Reuse</p> |
|  <p>Contains Sufficient for Test</p> |  <p>This Way Up</p> |

Safety, Quality, and Disposal

Safety

For professional use in a laboratory environment only.

- Ensure proper training on all instruments prior to use. Follow manufacturer instructions and perform quality control checks before each experiment.
- Operate in accordance with regulations for PCR gene amplification laboratories.

Warning: the oil-filled, ready-to-use RDG16 Cartridge is classified as hazardous according to Regulation (EC) No. 1272/2008 [CLP]:



Hazard Statements

H410: Very toxic to aquatic life

Precautionary Statements

P273: Avoid release to the environment.

P391: Collect Spillage.

P501: Dispose of contents/container in accordance with local, regional, national and international regulations.

Handle with Good Laboratory Practices (GLP).

- Do not eat, drink or smoke when using this product.
- Always wear appropriate personal protection equipment for handling this product - lab coat, disposable gloves, and appropriate eye/face protection are required. Whenever needed, wear additional personal protection equipment.
- Contaminated work clothing should be removed immediately and not be allowed out of the workplace until decontaminated. Change disposable gloves frequently and to prevent cross-contamination.
- Prevent release of oil or oil-contaminated components into the environment.
- Before breaks and after work, always wash your hands.
- All materials of human origin are to be treated as potentially infectious. Handle samples based on Standard and Universal Precautions, following local, regional, and national guidelines (such as Biosafety in Microbiological and Biomedical Laboratories). Dispose of all samples according to biohazardous and medical waste management regulations.
- Handle with general biosafety laboratory ventilation.

In case of exposure:

- General information: Call a doctor/physician or POISON CENTER if you feel unwell.
- In case of skin contact, wash with soap and water. If skin irritation or a rash occurs, seek medical advice/attention.
- In case of eye contact, rinse continuously with water for several minutes. Remove contact lenses if present

and easy to do. If symptoms persist, consult an ophthalmologist.

- If inhaled, provide fresh air.
- If swallowed, rinse the mouth.
- Self-protection of the first aider: no special measures are necessary
- In case of fire, use water, foam, or another agent suitable for ordinary combustibles.
- Clean up all spills immediately and thoroughly. Clean oil spills using a cleaning wipe or inert absorbent material. Dispose of contaminated materials according to the Disposal section.

Refer to the Safety Data Sheets (SDS) for more safety information. The Safety Data Sheets are available at bio-rad.com and on request (contact Bio-Rad Technical Support).

Quality Control

Each RDG16 package is tested according to EN ISO 13485:2016. A Certificate of Compliance is available upon request from Bio-Rad Technical Support.

Disposal

Dispose of all kit components and contaminated materials appropriately and per all pertinent regulations.

Waste oil or items contaminated by it must be disposed of as special or hazardous waste. It must NOT be released into the environment.

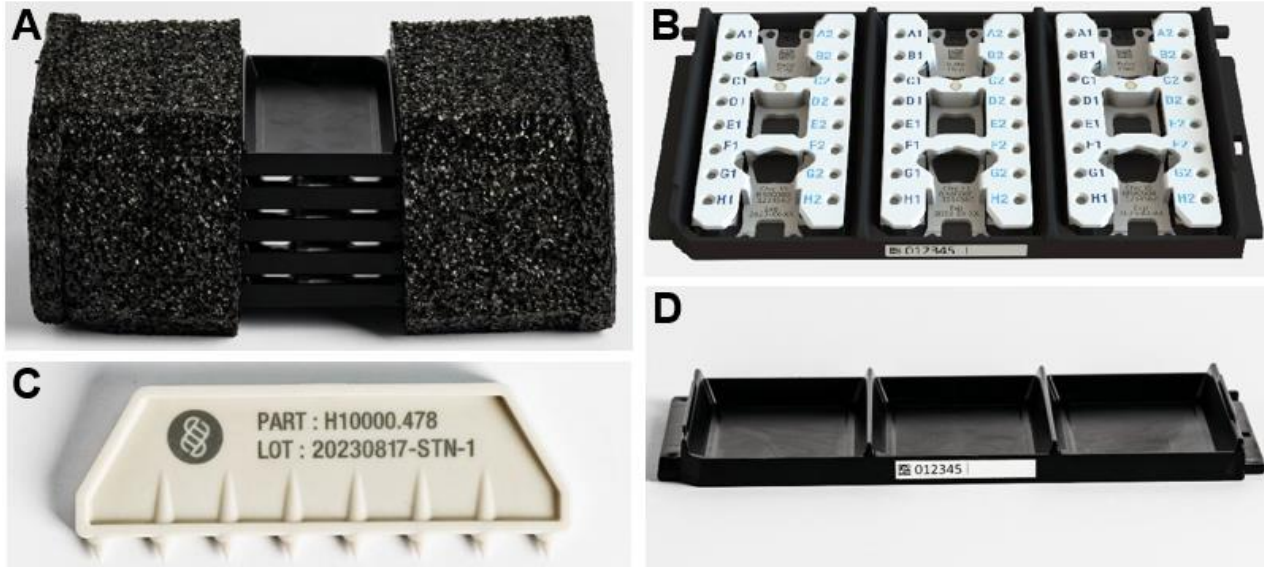
Waste that is considered biohazardous must be disposed of according to the requirements applicable to your laboratory or location.

For recycling of cardboard packaging, follow the requirements applicable to your laboratory or location.

Contents

Each RDG16 box contains an inflated, thermally sealed aluminum pouch. Inside the pouch are five RDG48 Plates (Figure D), stacked and wrapped in protective foam (Figure A).

- Each of the four lower RDG48 Plate holds three RDG16 cartridges, for a total of 12 cartridges (Figure B)
- The top RDG48 Plate contains a Piercing Tool enclosed in a plastic bag (Figure C).



Requirements and Compatibility

Instrument Compatibility

RDG16 Cartridges are compatible with the QX700 ddPCR System, the Nio Digital PCR system, and the naica system.

Master Mix Compatibility

Table 1 displays the ddPCR master mixes with which the RDG16 Cartridge has been fully verified and validated for optimal technical compatibility.

Table 2: Master mixes compatible with the cartridge

| Description | Supplier | Part Number |
|-------------------------------|----------|--------------------|
| naica Multiplex ddPCR Mix 10x | Bio-Rad | 12025255, 12025256 |
| naica Multiplex ddPCR Mix 5x | Bio-Rad | 12025254, 12025253 |
| naica ddPCR Mix 10x | Bio-Rad | 12025258 |
| naica ddPCR Mix 5x | Bio-Rad | 12025257 |

RDG16 Cartridge is compatible with:

- naica ddPCR Mix for EvaGreen® dye detection on the QX700 ddPCR system, the Nio Digital PCR system, and the naica system
- naica ddPCR Mix with Crystal Universal Reporters for Crystal Flex Probes detection on the Nio Digital

PCR system and the naica system

- naica multiplex ddPCR Mix for dual-labeled fluorescent probes (e.g. TaqMan probes) on the QX700 ddPCR System, the Nio Digital PCR system, and the naica system.

Storage and Inspection upon Delivery

Follow the instructions below to ensure the RDG16 Cartridges are intact, complete, and ready for use upon delivery. Check for any damage or missing components immediately upon delivery before proceeding.

- Do not use the product if the inflated pouch is pierced or deflated. Contact Bio-Rad Technical Support if the pouches are pierced or deflated.
- Do not use the product if any plates appear damaged. Contact Bio-Rad Technical Support if the cartridges or plates appear damaged.
- If the box is delivered upside down but the packaging (including the pouch) is intact, the cartridge remain usable. Reorient the box according to the arrows printed on it.
- Store the RDG16 Cartridge box in a clean area at room temperature (+15°C to +25°C) and standard humidity (20–55% in controlled laboratories).
- Keep unopened boxes in the upright position, as indicated by the arrows on the box.
- Store in a well-ventilated area, away from heat sources, strong bases, alkalis, and oxidizing agents
- Do not use cartridges after the expiration date printed on the box and on each cartridge.

Opening the Package and Storage after Opening

Follow the instructions below to ensure the RDG16 Cartridges are intact, complete, and ready for use upon delivery. Check for any damage or missing components immediately upon delivery before proceeding.

To open the inflated pouch

1. Keep the cartridge box upright, as indicated by the arrows printed on the packaging.
2. Remove the inflated pouch from the box and maintain the orientation shown on the label.
3. Use scissors to cut open the inflated pouch.

To open the RDG48 Plate package

1. Remove the protective foam surrounding the five plates.
2. Discard the top (empty) plate to access the four plates containing the cartridges.

After Opening the Pouch

- Store the cartridges in their plates inside the original box.
- Discard the inflated pouch and protective foam.
- Keep the plates upright at all times.



Figure 3: Cartridges on the plate

Usage Requirements

- Handle the cartridges by the side handles only. Avoid touching the underside to prevent compromising analysis.
- Keep the cartridges upright, with inlet ports facing upward, to prevent oil leakage.
- Keep the cartridges in their plates during loading, transport, and storage (including after ddPCR):
 - **naica system** – from loading to the Geode, and from the Geode to the Prism3 or Prism6.
 - **QX700 ddPCR System and Nio Digital PCR system** – Cartridges must be on the plate when loading into the instrument.
- Plates are made of conductive plastic to protect against electrostatic discharge and reduce ddPCR coalescence.
- If using fewer than three cartridges per run, use the spare plate provided in the box.
- Each plate can be reused up to three times. For more information, see the Plate Cleaning section.
- Each cartridge has a barcode for scanning and space for manual labeling.
- Wipe the bottom of each cartridge with an anti-static wipe (e.g., ACL Staticide, Ref: SW12) before use.
- Pierce all inlet ports using the provided Piercing Tool before starting the run, regardless of how many wells are loaded.
- Start the ddPCR run within 8 hours of loading the samples:

Note : Include any instrument wait time in this limit.

Note: For sensitive samples or applications, load cartridges immediately before starting the run.

ddPCR Guidelines

To maximize efficiency, amplicons should ideally be no longer than 130 bp in length.

Assay performance might be impaired with longer amplicons, particularly when using highly fragmented DNA templates (for example, FFPE DNA or circulating DNA).

DNA Digestion Restriction

To ensure even distribution of the DNA template during partitioning, DNA samples with ≥ 10 kb average length (for

example, genomic DNA) should be fragmented by restriction digestion before partitioning. Digestion restriction is not required for highly fragmented DNA (for example, FFPE DNA or circulating DNA), but you must use restriction enzymes that do not cut within the amplified sequence.

DNA fragmentation by digestion restriction is important in various applications and particularly important in copy number variation (CNV) analyses.

Caution: Input material for the ddPCR workflow includes extracted nucleic acid. The purity of the extracted sample may vary depending on the raw material and the implemented extraction protocol. Bio-Rad recommends performing validation tests to select a compatible extraction protocol for optimal ddPCR performance.

Sample Preparation

Loading samples into cartridges involves three main steps:

1. Applying anti-static treatment to the bottom of the cartridges
2. Piercing the inlet ports
3. Pipetting the samples

Applying Anti-Static Treatment

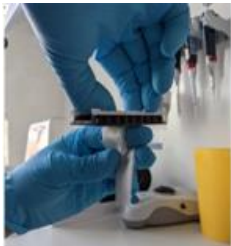
To apply anti-static treatment

4. Place an RDG16 Cartridge in a RD48 plate on a laboratory bench.



Note: If using fewer than three cartridges, place only the required number of cartridges in the additional RDG48 Plate provided in each cartridge box. Leave the remaining cartridges in their original plate.

5. Open a pouch of the pre-wetted anti-static wipe (ACL Staticide, part number: SW12).
6. Wrap the wipe around a finger and thoroughly wipe the underside and edges of the cartridge to fully coat it with anti-static treatment.



7. Without turning the cartridge upside down, lift the cartridge and visually check that the entire bottom surface is evenly coated with anti-static solution.
8. Wrap a piece of Precision Wipe (Kimtech Science, part number: 7552) around a finger and gently wipe the bottom of the cartridge to remove any excess anti-static solution.



9. Place the cartridge back into the plate and repeat Steps 2–4 for the next cartridge.

Note Use one ACL Staticide wipe for up to three cartridges, if treated consecutively.

Note: If the wipe dries out, use a new one.

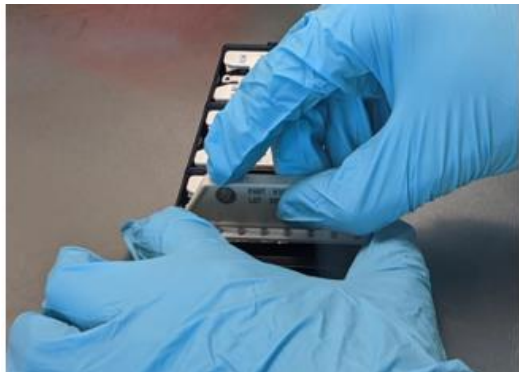
10. Keep the cartridges in the plate and proceed to the inlet port piercing step.

Piercing the Cartridge Port

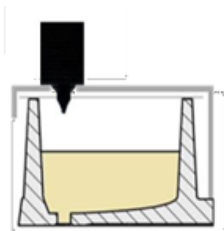
Important: Piercing the Ruby Chip with the piercing tool is mandatory when operating at altitudes above 500 meters.

To pierce the port

1. Hold the Piercing Tool in one hand. With the other hand, secure the cartridge in the plate by placing your index or middle finger at the top of the cartridge and your thumb at the bottom.



2. Position the Piercing Tool above a column of inlet ports and in contact with the aluminum foil cover.
3. Press the Piercing Tool down firmly to pierce the foil.



Pipetting the Sample Mix

This section describes how to pipette the sample mix into the cartridge using the following methods:

- Single-channel pipette
- Multi-channel pipette (eight channels)
- Pipetting robot

Follow the appropriate procedure based on your lab's setup to ensure accurate and consistent loading.

Note: Bio-Rad recommends using universal 10 μ L or 20 μ L pipette tips for sample mix injection. Avoid using positive displacement pipettes, LTS tips (LiteTouch tip ejection system), or wide-range volume tips (e.g., 1–100 μ L), as they may compromise loading accuracy. For questions about tip compatibility, contact Bio-Rad Technical Support.

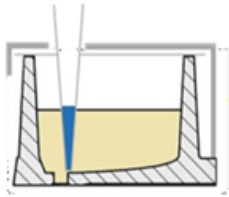
Sample Mix Loading: Single-Channel Pipetting

To pipette samples into the cartridge

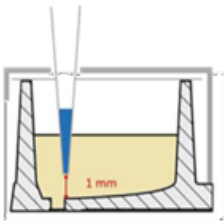
1. Load 5 μ L of sample mix into the pipette tip.
2. Hold the pipette in one hand. With the other hand, secure the cartridge in the plate by placing your index or middle finger at the top of the cartridge and your thumb at the bottom.



3. Hold the pipette vertically (at a 90° angle) above the inlet port.
4. Insert the pipette tip into the inlet well and gently touch the bottom.

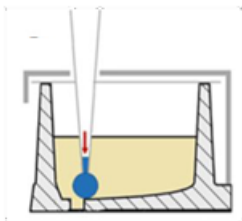


5. While maintaining a 90° angle, lift the tip about 1 mm from the bottom so that it remains submerged in the oil.



6. Keeping the pipette steady, eject the sample mix by pressing the plunger to the first stop only, in one continuous and firm motion.

Important: Do not press pipette plunger to the second stop to avoid introducing air bubbles.



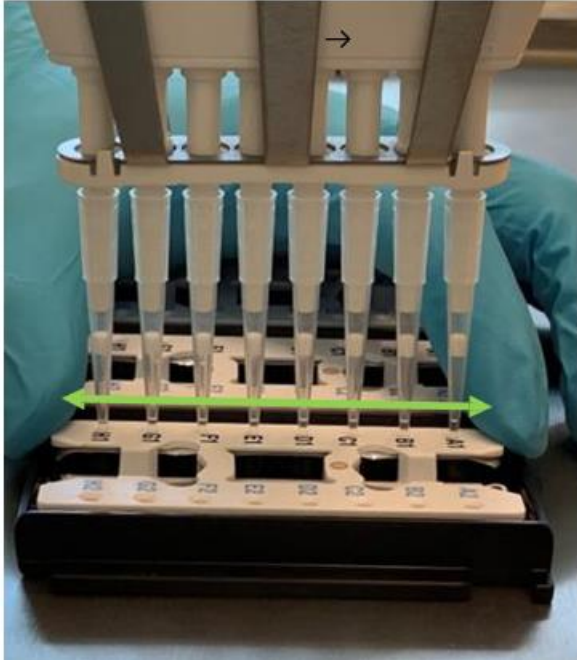
7. Withdraw the pipette tip slowly and vertically from the inlet well.

Note: Use a new pipette tip for each inlet port (even when using the same sample mix) to prevent cross-contamination and bubble formation.

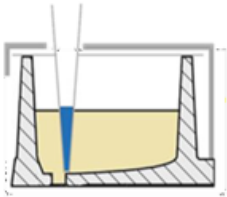
Sample Mix Loading: Multi-Channel Pipetting

To pipette samples into the cartridge

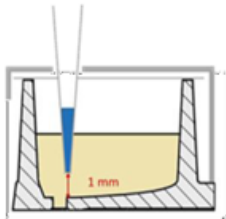
1. Load 5 μL of sample mix into each of the 8 tips of the multichannel pipette.
2. Hold the pipette in one hand. With the other hand, secure the cartridge in the plate by placing your index or middle finger at the top of the cartridge and your thumb at the bottom.



3. Hold the pipette vertically (at a 90° angle) above the eight inlet ports.
4. Insert the eight pipette tips into the inlet wells and touch the bottom of each well.



5. Maintaining the 90° angle, gently lift all tips about 1 mm above the bottom so they remain submerged in oil



Important Ensure all eight tips are at the same height before pipetting.

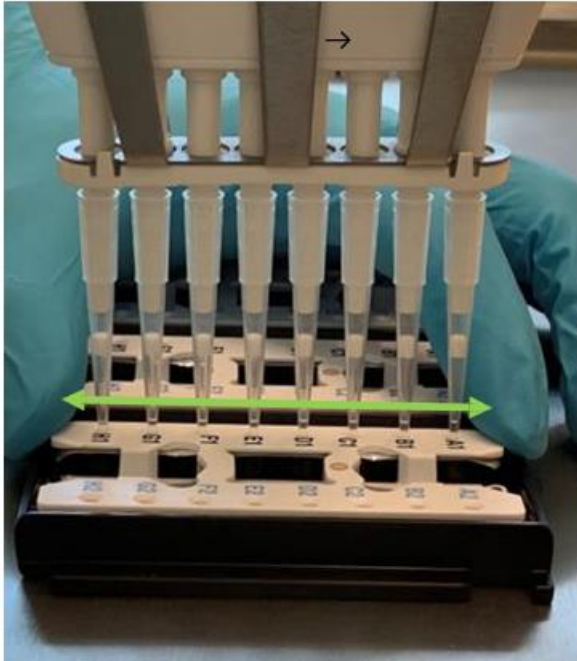


Figure 4: Correct positioning of an 8-channel pipette for sample injection

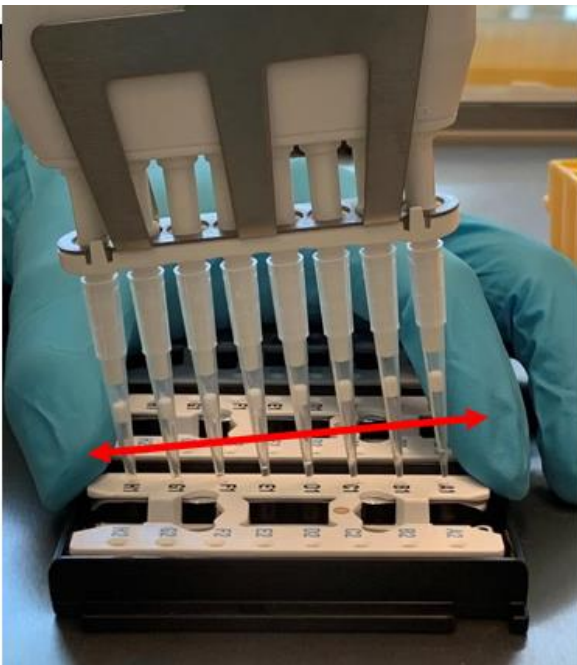


Figure 5: Incorrect positioning of an 8-channel pipette for sample injection

6. Keeping the pipette steady, eject the sample mix by pressing the plunger to the first stop only, in one continuous and firm motion.

Important: Do not press pipette plunger to the second stop to avoid introducing air bubbles.

7. Withdraw the pipette vertically and slowly from the inlet wells.

Pipetting with an Automated Pipetting System or Robot

The RDG16 cartridges are compatible with most automated pipetting systems.

For guidance on setup and integration, refer to the technical note: “Product Demonstration – Integration of Automated Liquid Handling of RDG16 Cartridge Enables a Fully Integrated Workflow Solution for High-Throughput Crystal Digital PCR.”

For additional support, contact Bio-Rad Technical Support.

Partitioning and Amplification at High Altitudes

Partitioning and Amplifying with the naica System

When partitioning and amplifying with naica System at elevations above 500 meters, partitioning and amplification using the RDG16 pate require modifications to the standard Geode PCR programs provided in the user manual.

Use the appropriate Altitude RDG16 Cartridge PCR Templates listed below.

DNA Applications

Use the ddPCR™ template called “Template Altitude ddPCR 45 cycles”. Use naica PCR reagents (naica PCR MIX, naica multiplex PCR MIX) with the RDG16 Cartridge.

The total duration of the default PCR program is approximately 2 hours and 15 minutes

| Step | Temperature | Pressure * (mbar) | Duration ** |
|-----------------------------|-------------|-------------------|-------------|
| Partition | 25°C * | AP to +650 | 55 minutes |
| Initial denaturation | 95°C | +600 | 3 minutes |
| PCR (45 cycles) | 95°C | +600 | 10 seconds |
| | 55-65°C | +600 | 15 seconds |
| Release | Down to 25 | Down to AP | 2 minutes |

* Do not modify

** Duration values are indicative; variances can occur.

RNA Applications

Use the ddPCR for RT-PCR template called “Template Altitude Ruby RT-PCR 45 cycles.”

Use Quanta Biosciences qScript™ XLT One-Step RT-qPCR ToughMix with the RDG16 Cartridge

The total duration of the default PCR program is approximately 2 hours and 15 minutes.

| Step | Temperature | Pressure * (mbar) | Duration ** |
|----------------------|-------------|-------------------|-------------|
| Partition | 25°C * | AP to +650 | 55 minutes |
| cDNA synthesis | 50°C | +600 | 10 minutes |
| Initial denaturation | 95°C | +600 | 1 minute |
| PCR (45 cycles) | 95°C | +600 | 30 seconds |
| | 95°C | +600 | 15 seconds |
| Release | Down to 25 | Down to AP | 2 minutes |

* Do not modify

** Duration values are indicative; variances can occur.

Notes:

- The PCR and RT-PCR templates described above are not preloaded in the Geode. They must be manually imported.
- Using the altitude-specific programs may lead to inaccurate remaining time estimates on the Geode user interface.
- PCR program durations may slightly vary across different Geode instruments due to intrinsic thermal plate variations.

Partitioning and Amplifying with the QX700 ddPCR System or the Nio Digital PCR System

At altitudes above 500 meters, you must adjust the partitioning step when using RDG16 Cartridges. The QX700 ddPCR System Control Software includes an altitude-adapted partitioning protocol.

To enable the high-altitude protocol:

1. Open the QX700 ddPCR System Control Software (QX700 ddPCR System) or Nio Reader software (Nio Digital PCR system) and navigate to the Protocol Page.
2. In the Thermocycling Program editor, select the Partition step.
3. Select the Adapt for altitude >500m check box (QX700 ddPCR System) or Adapt for high altitudes check box (Nio Digital PCR system).

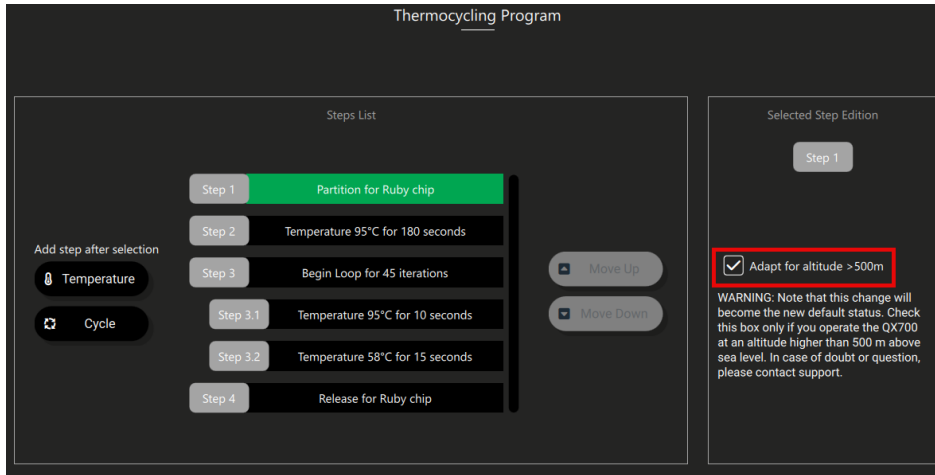


Fig 6. QX700 ddPCR System



Fig. 7 Nio Digital PCR System

Note: The software automatically applies the “Adapt for high altitude” setting to the Release step as well.

Cleaning and Reusing the RDG48 Plate and Piercing Tool

Cleaning the RDG48 Plate

Each RDG48 Plate holds up to three RDG16 Cartridges. If you're using fewer than three cartridges, reuse the plate as a spare. You can reuse each cartridge up to three times, as long as you clean and decontaminate it between runs to prevent contamination.



Figure 6: RDG48 Plate with one RDG16 Cartridge

To clean and decontaminate a plate:

1. Remove all cartridges.
2. Spray ethanol on a lab wipe and clean all inner and outer surfaces.
3. Spray a decontaminant (e.g., RNase AWAY) on a new wipe and repeat the cleaning.
4. Let the plate air dry completely before reuse

Note: You can wipe the front label with ethanol to relabel it. The QR code and plate number will remain intact. Use a pen or marker to write on the label again.

Cleaning the Piercing Tool

Bio-Rad recommends cleaning the piercing tool before each PCR run to prevent contamination.

To clean the Piercing Tool:

1. Spray a lab wipe with a decontaminant such as RNase AWAY.
2. Hold the piercing tool in one hand and the wipe in the other.
3. Wipe the row of piercing cones from base to tip to avoid leaving fibers on the cones.



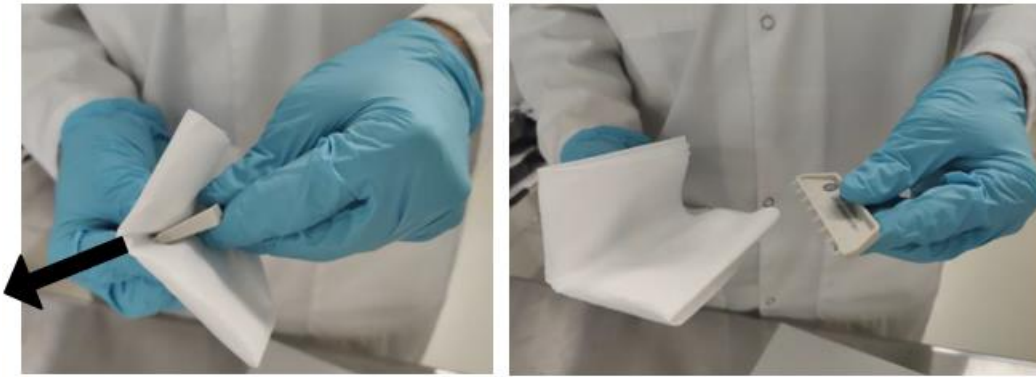


Figure 7: Cleaning the piercing tool

Note: Between experiments, store the piercing tool in its transparent storage bag to protect it from dust and RNA/DNA contamination.

Troubleshooting

- Interpret results from wells with fewer than 10,000 droplets with caution. Closely examine the ddPCR data for possible artifacts that could affect quantification.
- Contact Bio-Rad Technical Support for any cartridge-related issues.

Consumables Required but Not Provided

- Reagents and consumables for nucleic acid purification
- Standard consumables and equipment for PCR mix preparation
- Assay-specific digital PCR reagents, primers, and probes
- Antistatic wetted wipes (ACL Staticide, Ref: SW12)
Note: This product is available from Digi-Key (Ref: ST1059-ND)
- Precision wipes (Kimtech Science, Ref: 7552, 1-ply, 213 × 114 mm)

Note: This product is available from standard laboratory supplier

Contacting Technical Support

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

Phone: 1-800-424-6723, option 2

E-mail: Support@bio-rad.com (U.S./Canada Only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at www.bio-rad.com.

Documentation

- QX700 Droplet Digital PCR System Instrument Guide (DIR No. 10000171493)
- QX700 ddPCR System Analysis Software User Guide (DIR No. 10000171494)

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Stilla Technologies
F-94800 Villejuif, France



Bio-Rad Laboratories, Inc.
1000 Alfred Nobel Drive
Hercules, CA, 94547 USA