



## Crystal Digital PCR Assays for use with Crystal Universal Reporters

### Instructions for Use

February 2026

*For research use only. Not for use in diagnostic procedures.*

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## Purpose

This document provides information and instructions for using a Crystal Digital PCR Assay with Crystal Universal Reporters and is intended for laboratory personnel who are trained to perform digital PCR (dPCR) procedures.

Important: The dPCR assays specified herein, and their associated components, are intended for research use only (RUO). *Do NOT use them for diagnostic procedures.* Laboratory personnel and other users must complete all required training before using dPCR systems.

## Intended Product Use

Crystal Digital PCR Assays are intended for use with Crystal Universal Reporters, which are ordered separately. They are compatible with all Nio Digital PCR instruments, as well as the 6-color and 3-color naica systems, provided that the chosen Crystal Universal Reporters match the detection channels of the instrument, as specified in Table 1 below.

**Table 1 Crystal Universal Reporter color and imaging channel compatibility by dPCR instrument**

Reporter	Reporter detection color	Compatible Channels		
		Nio <sup>1</sup>	Prism6	Prism3
Crystal Universal Reporter, Tube A	Blue	✓	✓	✓
	Green	✓	✓	✓
	Red	✓	✓	✓
Crystal Universal Reporter, Tube B	Teal	✓	✓	None
	Yellow	✓	✓	None
	Infra-Red	✓	✓	None
	Long-Shift	✓	None	None

Note the following:

- Crystal Digital PCR Assays are available in 200 and 1000 reaction formats.
- The assays are wet lab-validated on synthetic DNA.
- Positive controls are available and included with the assay.
- Individual sample-type and extraction method compatibilities for dPCR applications might require a dedicated validation by the end user.
- Crystal Digital PCR Assays might require additional optimization and validation by the end user.
- Sample matrices might impact the assay performance.

<sup>1</sup> Applies to all Nio instrument configurations

## About Crystal Digital PCR Assays with Crystal Universal Reporters

Crystal Digital PCR Assays allow for DNA and RNA quantification of specific targets using Crystal Flex Probes and Crystal Universal Reporters.

Crystal Flex Probes are non-fluorescent oligonucleotides that are specific to the target of interest. They contain a non-target specific sequence, which is a tag oligonucleotide that triggers the fluorescence of the complementary Crystal Universal Reporter when released during dPCR.

Crystal Universal Reporters generate fluorescent signals when activated during a dPCR reaction using the exclusive target-specific Crystal Flex Probe detection chemistry. Their universal design allows their use with any Crystal Digital PCR Assay, which provides a modular approach to detection. You can use the same set of Crystal Universal Reporters to detect and quantify all targets of interest, regardless of the specific assay or the degree of multiplexing. When higher order multiplexing is required to detect more targets than fluorescent channels available in a single reaction, dPCR assays use color combinations. Using the Crystal Universal Reporters with color combination high-plex assay design makes detecting more than seven targets possible in a single reaction with a streamlined 1D thresholding analysis.

A Crystal Digital PCR Assay is provided in a ready-to-use, assay-specific mix that also contains dPCR primers and Crystal Flex Probes. The mix is optimized for use with the naica 10X ddPCR Mix (Catalog No. 12025258) and RDG16 cartridges<sup>2</sup> (Catalog No. 12025252), which are ordered separately. The list of Crystal Digital PCR Assays is available from the [Nio System Product Page](#) and the [naica System Product Page](#).

### Crystal Digital PCR Assay Composition

A Crystal Digital PCR Assay package contains the components listed in Table 2, which are available in 200 or 1000 reaction formats.

**Table 2. Assay composition**

Component Name	Cap Color	Init Conc	Volume 200/1000 rxn	Description
Crystal Digital PCR Assay	Orange	10x	120/600 µL	Ready to use primer and probe solution based on Crystal Flex Probe technology
Positive Control	Clear	10x	120 µL	Synthetic DNA pool

**Note:** For details on the targets included in the positive control, refer to the assay-specific Information Sheet.

<sup>2</sup> RDG16 cartridge product references are replacing the Ruby Chip product references. RDG16 cartridges feature the same formulation and performance as Ruby Chip.

## Use and Safety Requirements

**Important:** Before using this product, review the Crystal Universal Reporters Safety Data Sheet (SDS), and comply with all specified requirements, hazard statements, and environmental precautions to ensure personal, operational, and environmental safety. You can download the document after logging into [bio-rad.com](http://bio-rad.com) or you can contact Bio-Rad Technical Support.

### Safety Precautions and Recommendations

The products referenced herein are intended for professional use in a laboratory environment only.

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

Before using this product, review the Safety Data Sheet and comply with all requirements, hazard statements, and environmental precautions to ensure personal, operational, and environmental safety. You can download the document after logging into [bio-rad.com](http://bio-rad.com), or you can contact Bio-Rad Technical Support.

Crystal Digital PCR Assays and Crystal Universal Reporters are NOT classified as dangerous according to Regulation (EC) No. 1272/2008 [CLP]. However, to handle this product you must wear appropriate personal protection equipment, such as disposable gloves, lab coat, and goggles or safety glasses, and wear additional personal protection equipment when necessary. Additional recommendations are specified below:

- Wash hands before breaks and after work.
- Remove and properly dispose of contaminated, saturated clothing.
- Avoid release of oil or components contaminated with oils into the environment.
- Sample handling and waste disposal must meet relevant regulatory requirements.
- If there is a spill, use a cleaning wipe and then dispose in accordance with the applicable regulations.
- Ensure appropriate ventilation.

## Materials Required but Not Provided

- Crystal Universal Reporters, as shown in Table 3; use Crystal Universal Reporter 3 (Tube A) for up to three-color multiplexing, and use Crystal Universal Reporter 7 (Tube A and Tube B) for higher multiplex levels.

**Table 3. Crystal Universal Reporters products for Crystal Digital PCR Assays**

Catalog No.	Product Name	Cap Color	Initial Conc	Volume 200/1000 rxn	Description
12026172 12026157	Crystal Universal Reporter 3	Tube A Yellow	40x	30 $\mu$ L 150 $\mu$ L	Ready to use reporter solution for detection in the blue, green, and red channels of Prism3, Prism6, and Nio
12026173 12026136	Crystal Universal Reporter 7	Tube A Yellow	40x	30 $\mu$ L 150 $\mu$ L	Ready to use reporter solution for detection in the blue, green, and red channels of Prism3, Prism6, and Nio
		Tube B Brown	40x	30 $\mu$ L 150 $\mu$ L	Ready to use reporter solution for detection in the teal, yellow, and Infra-red channels on Prism6 and Nio and the long-shift channel on Nio  <b>Note:</b> Long-shift is available on Nio only.

- Nio Digital PCR System (all configurations), or naica system (6-color or 3-color)
  - naica 10X ddPCR Mix (Cat. #12025258)
  - RDG16 cartridges (Cat. #12025252)
- Note:** RDG16 cartridges are replacing the discontinued Ruby Chip product.
- DigiKey ACL Staticide antistatic wetted wipes (DigiKey catalog no. ST1059-ND SW12)
  - Precision Wipes (Kimtech™ Science, Reference: 7552, 1 ply, 4.4 in x 8.2 in (114 mm x 213 mm); available from general suppliers.
  - Standard consumables and equipment for PCR reaction mix preparation:
    - PCR-compliant reaction tubes
    - Centrifuge for microcentrifuge tubes (~700xg)
    - Laboratory mixer for vortexing
    - Micropipettes
    - Micropipette tips
    - Nuclease-free water

## Handling and Storage Requirements

Crystal Digital PCR Assays and Crystal Universal Reporters are shipped on dry ice.

**Important: Immediately upon receipt**, inspect package integrity and ensure the correct storage of the Crystal Digital PCR Assay and Crystal Universal Reporter at the indicated storage temperatures. If there is any doubt regarding the integrity or correct storage of the assay, do NOT use the assay and contact Bio-Rad Technical Support.

Under the following conditions, the Crystal Digital PCR Assay and Crystal Universal Reporters are stable until the expiration date indicated on the box:

- You must store all reagents provided in the Crystal Digital PCR Assay and Crystal Universal Reporter boxes as follows:
  - At -20° C, ± 5° C
  - In the original tubes
  - Until the expiration date specified on the packaging

**Important:** Do NOT aliquot in alternative tubes.

- Avoid multiple freeze/thaw cycles. Reagents can be thawed up to 10 times without observable deviations in performance
- Store tubes away from light sources
- At all times, store tubes in an upright position
- Ensure all tube caps are well-closed when not in use or before returning to storage

## Disposal Requirements

Dispose of all package components and contaminated materials appropriately and per all pertinent regulations. To recycle cardboard packaging, follow the requirements applicable to your laboratory or location.

## Product Resources

You can obtain detailed information on the detected targets, positive controls, specific thermocycling program, scanning settings, analysis configuration, and representative data in the Information Sheet of each Crystal Digital PCR Assay. Technical resources for Crystal Digital PCR Assays can be accessed at [bio-rad.com](http://bio-rad.com).

Before you begin, read the instrument manuals for the Nio and naica systems, and the Instructions for Use for the naica 10X ddPCR Mix (Catalog No. 12025258) and RDG16 Cartridges (Catalog No. 12025252).

## Best Practices

### Digital PCR

For optimal dPCR efficiency using Crystal Flex Probes, amplicons should (ideally) be no longer than 130 bp. Assay performance might be impaired with longer amplicons, particularly when using highly fragmented DNA templates (for example, FFPE DNA or circulating DNA).

**Important:** Input material for the dPCR workflow includes extracted nucleic acid. The purity of the extracted sample may vary depending on the raw material and the implemented extraction protocol. It is recommended to perform validation tests to select a compatible extraction protocol for optimal dPCR performance.

### DNA Digestion

Before partitioning, DNA samples with  $\geq 10$  kb average length such as genomic DNA) can be fragmented using restriction digestion to ensure even distribution of the DNA template during partitioning. This step might improve assay performance and should be tested utilizing desired samples.

**Important:** Take care to use restriction enzymes that do not cut within the amplified sequence or the Crystal Flex Probes.

For a list of restriction enzymes compatible with a given Crystal Digital PCR Assay, contact Bio-Rad Technical Support.

Restriction digestion is not required for highly fragmented DNA.

### DNA Concentration

For optimal performance, do NOT exceed a chamber concentration (DNA concentration in the reaction mix) of 1000 copies/ $\mu$ L. For example, the corresponding human DNA to a DNA concentration in the reaction mix is 3.3 ng/ $\mu$ L. You can determine correspondence between copies and DNA using the genome size of the species being analyzed and calculators that are available on the internet.

## Protocol

### Prerequisites

Complete the steps in this section to prepare and load the reaction mix.

1. Operate the Crystal Digital PCR Assay in the 20° C to 25° C temperature range:
2. Before you prepare the reaction mix, completely thaw all reagents listed below:
  - Crystal Digital PCR Assay
  - Crystal Universal Reporters
  - Positive Control (recommended for inclusion in test plan to optimize thresholding)
  - naica 10X ddPCR Mix

**Note:** For DNA applications, use the Naica 10X ddPCR Mix. For RNA applications, refer to the dedicated Assay Information Sheet for the recommended RT PCR supermix.

3. Vortex all tubes 10 sec at maximum speed and spin briefly in a mini-spin centrifuge to collect the material at the bottom of each tube.
4. Prepare a reaction mix according to the applicable Assay Information Sheet.
5. Vortex and spin down the PCR reaction mix.
6. Dispense appropriate volumes of the reaction mix into standard PCR tubes or plates. The reaction mix contains all components except the template DNA.
7. Add the template DNA (samples, positive control, or negative control) to each tube or well according to the test plan.

**Important:** To limit the risk of contamination, close each tube after DNA template and prepare the positive control last.

8. Vortex the tubes thoroughly to mix all reagents, and then briefly centrifuge to collect the content at the bottom of the tubes.
9. Load 5 µL of reaction mix per chamber in the RDG16 cartridge, refer to the IFU for information.
10. Perform the dPCR reaction. If you are using the Nio instrument, continue to the next section (Using the Nio Digital PCR System). If you are using the naica system, skip to the subsequent section (Using the naica System).

## Using the Nio Digital PCR System

Download the Assay Information Sheet for the desired Crystal Digital PCR Assay to obtain information on its thermocycling protocol and scanning parameters from [bio-rad.com](http://bio-rad.com). For instructions on how to run a Crystal Digital PCR Assay experiment with the Nio system, and how to analyze results in the Nio Analyzer software, refer to the respective instrument or user guide. For each Crystal Digital PCR Assay, the .nioprotocol and .nioassay files are also available for download. See [Nio System Product Page](#).

### To run an experiment

1. Open the Nio Reader software.
2. Under DIGITAL EXPERIMENTS, tap/click New and then tap/click Start from a blank experiment.
3. In the Files pane, select the Protocol tab and do the following:
  - a. Click Add file.
  - b. Browse to the .nioprotocol file.
  - c. Select the file and tap/click Open.

The file appears in the Protocols tab.

4. In the Files pane, select the Assay tab and do the following:
  - a. Click Add file.
  - b. Browse to the .nioassay file.
  - c. Select the file and tap/click Open.

The file appears in the Assays tab.

**Note:** The .nioassay file embeds the compensation matrix and analysis file.

5. In the Chip Plate Layout pane, modify the sample name chamber and drag and drop the .nioprotocol and .nioassay from the Files pane to the Chip Plate layout pane according to the experiment plan.
6. In the upper-right corner of the screen, select Save As and enter a file name for the experiment file.

**Tip:** You can use the saved .nioexperiment file as a template for future experiments with the same experiment setup.

7. Under RUNS, select Plan & Start and then browse and load the .nioexperiment file you saved in previous step.
8. Load the RDG16 cartridges into the Nio instrument.
9. Select Start Run.

10. Under RUNS, tap/click Status to verify that the cartridges enters the thermal cycling process.

If there are previously inserted cartridges in progress, your cartridges are queued. Scanning occurs when thermal cycling is complete and a quality flag appears after a chamber is scanned. Refer to the Nio Reader User Manual for detailed instructions to check image quality.

11. When the run concludes, copy the .niodata results file to a USB drive.
12. To recover the cartridges, tap/click Status under RUNS, and select the dPCR run to unload.
13. Tap/click Unload cartridges on the right.

**Note:** If necessary, RDG16 cartridges can be rescanned.

## Using the naica System

Download the Assay Information Sheet specific to the desired Crystal Digital PCR Assay for details on its thermocycling protocol and scanning parameters. For each Crystal Digital PCR Assay, Scanning Template files are also available for download from the [naica System Product Page](#).

For instructions on how to run a dPCR experiment with the Geode thermal cycler and the Prism3 or Prism6 color reader, refer to the respective user manuals. Refer to the Crystal Reader User Manual for instructions on how to acquire images using the Prism3 or Prism6 instrument.

### To run an experiment

1. Place the RDG16 cartridges in the Geode and close the lid.
2. Select the Crystal Digital PCR Assay-specific PCR program from the main menu of the Geode screen and press Play to start the program.
3. At the end of the run, the message *PCR completed successfully, touch the screen to continue* appears.
4. Turn on the Prism3 or Prism6.
5. Open the Geode lid and transfer the RDG16 cartridges to the Prism3 or Prism6.
6. Double-click the Crystal Reader icon on the monitor to launch the Crystal Reader software application.
7. To create a new experiment with the RDG16 cartridges, click NEW EXPERIMENT.
8. Select the scanning template file for the assay and in the prompt that appears, select Use template chamber details.
9. To name your experiment, fill in the NEW EXPERIMENT case and at a minimum, use the experiment type as a prefix. You can also add the date and cartridge IDs in the following format to maintain good searchability practices:  
  
    <Experiment type>-YYMMDD-<cartridge ID>
10. Enter the cartridge ID manually, or with an external barcode reader.
11. In the Chamber Details section, modify the sample name for each sample.
12. Click Open tray and place the RDG16 cartridges in the Prism3 or Prism6, and then click Close the tray.
13. Click Save and then navigate to the desired folder.
14. Click Scan.
15. After the scan is completed, dispose the RDG16 cartridges as per recommendations

**Note:** If necessary, RDG16 cartridges can be rescanned.

## Analysis of Results

1. Open the experiment results in the respective analysis software.
2. On the 1D plots for each channel, set thresholds to separate positive and negative droplets.

**Notes:**

- For some assays, the analysis is performed with polygons in 2D plots. Refer to the Assay Information Sheet of the specific Crystal Digital PCR Assay for additional instructions on threshold or polygon settings.
- The concentration of each target is shown in the View Results tab.
- Example data is provided in the Assay Information Sheet specific for each Crystal Digital PCR Assay.

## Symbols Lexicon

The following symbols appear on the product label.

 Manufacturer	 Distributor	 Catalog Number
 Batch Code	 Use By Date	 Temperature Limit
 If package is damaged, DO NOT USE (refer to IFU)	 Consult Instructions for Use	 Keep Dry
 Keep Away From Direct Sunlight	 Contains Sufficient for Test	 This Way Up

## Product Ordering Information

Catalog No.	Product Ref No.	Product Description
Multiple	Multiple	Primers and Crystal Flex Probes in 200 and 1000 reactions in RDG16 cartridge (see <a href="http://bio-rad.com">bio-rad.com</a> )
12026172	R41401	Crystal Universal Reporter 3, 200 rxn
12026157	R41402	Crystal Universal Reporter 3, 1000 rxn
12026173	R42401	Crystal Universal Reporter 7, 200 rxn
12026136	R42402	Crystal Universal Reporter 7, 1000 rxn

## Revision History

Release Date	SAP DIR and Version	Description
February 2026	10000255609 Ver A	Reformat/edit document using Bio-Rad branding and website hyperlinks

## Bio-Rad 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

**Email:** [Support@bio-rad.com](mailto: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 [bio-rad.com](http://bio-rad.com).

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