ddPLEX CrystalTag ddPCR™ Assays

For Use With QX700™ CrystalTag Reporters

Catalog#	Description
Multiple	ddPLEX CrystalTag ddPCR Assays (primers and CrystalTag Probes; see bio-rad.com)
12027210	QX700 CrystalTag Reporter 3 200R
12027271	QX700 CrystalTag Reporter 3 1,000R
12027244	QX700 CrystalTag Reporter 7 200R
12027265	QX700 CrystalTag Reporter 7 1,000R

For Research Use Only. Not for use in diagnostic procedures.

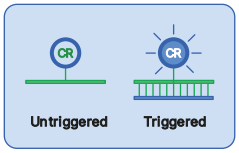
Description

ddPLEX CrystalTag Droplet Digital™ PCR (ddPCR) Assays allow for DNA and RNA quantification of specific targets using Bio-Rad's CrystalTag detection chemistry featuring two separately ordered components: 1) CrystalTag Probes and 2) QX700 CrystalTag Reporters:

- **CrystalTag Probes** are non-fluorescent oligonucleotides specific to the target of interest; each contains a non-target-specific tag sequence that triggers the fluorescence of the complementary QX700 CrystalTag Reporter when released during ddPCR



- **QX700 CrystalTag Reporters** generate fluorescent signals when activated during a ddPCR reaction. Their universal design allows them to be used with any ddPLEX CrystalTag ddPCR Assay, providing a modular approach to detection: the same set of QX700 CrystalTag Reporters can detect and quantify all targets of interest, regardless of the specific assay or degree of multiplexing



During amplification of the ddPCR reaction, a CrystalTag Probe bound to its target is cleaved, releasing its tag oligonucleotide. The released tag hybridizes to the complementary QX700 CrystalTag Reporter and activates its fluorophore, so fluorescence accumulates only in partitions that contain the target of interest.

Because the reporter components are independent of the target sequences, one set of QX700 CrystalTag Reporters serves every assay in the ddPLEX CrystalTag portfolio. Figure 1 illustrates the CrystalTag technology and how the probes and reporters work together.

ddPLEX CrystalTag ddPCR Assays are designed with CrystalTag technology following a color-combination high-plex design strategy, that allows detection of more than seven targets in a single reaction using a streamlined 1D thresholding analysis.

Product Purpose

Confirm these instructions for use match your ddPCR system.

- For Research Use Only. Not for use in diagnostic procedures.
- ddPLEX CrystalTag Assays are designed for use with QX700 CrystalTag Reporters to be used on the QX700 System.
- ddPLEX CrystalTag ddPCR Assays are wet lab tested on synthetic DNA. Sample matrix may have an impact on the assay performance. Individual sample-type and extraction method compatibilities for ddPCR applications may require dedicated verification testing by the end user. Positive controls are available and included with the assay
- Compatible with all QX700 Droplet Digital PCR Systems and RDG16 Cartridges

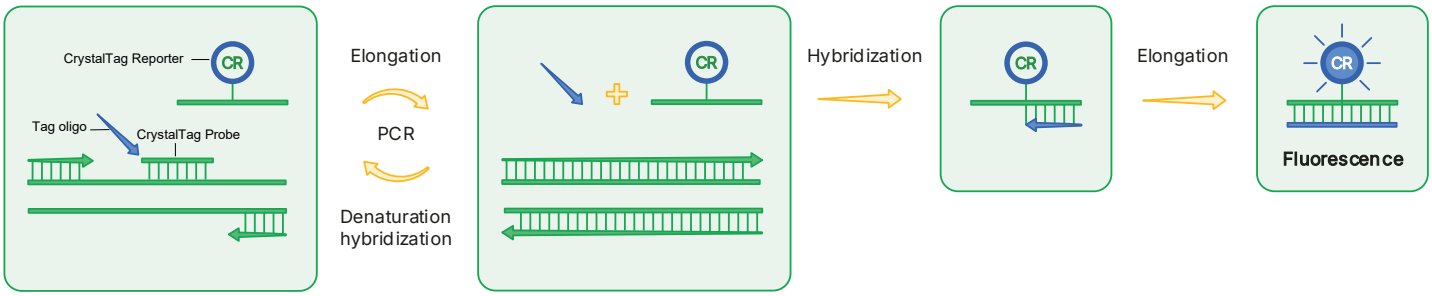


Fig. 1: How CrystalTag technology works. When the target of interest is present, CrystalTag Probes bind to the target. The tag oligonucleotide is cleaved and released to activate the CrystalTag Reporter and emit fluorescence.

Ordering Information

ddPLEX CrystalTag ddPCR Assays and QX700 CrystalTag Reporters are purchased separately.

- The list of available ddPLEX CrystalTag ddPCR Assays can be ordered online at **www.bio-rad.com/ddplex-crystaltag-ddpcr-assays**
- QX700 CrystalTag Reporters 3 and QX700 CrystalTag Reporters 7 can be ordered online at **www.bio-rad.com/ddplex-crystaltag-ddpcr-assays**

Detailed information on the detected targets, positive controls, specific thermocycling program, scanning settings, or analysis configuration, as well as representative data can be found in the Information Sheet of each ddPLEX CrystalTag ddPCR Assay.

Storage and Stability

ddPLEX CrystalTag ddPCR Assays and QX700 CrystalTag Reporters are shipped on dry ice. Immediately upon receipt, inspect package integrity; if there is any doubt regarding integrity or correct storage, do not use the product and contact Bio-Rad Technical Support.

Store all reagents at -20°C (± 5°C) in their original tubes until the expiration date on the packaging; do not aliquot into alternative tubes.

Store tubes upright and away from light and ensure all caps are well closed when not in use.

Avoid multiple freeze/thaw cycles. Reagents can be thawed up to 10 times without observable deviation in performance.

Safety and Disposal

Consult the Safety Data Sheet (SDS) for each component before use.

Handle all reagents with as potentially hazardous. Wear appropriate personal protective equipment, including gloves, a laboratory coat, and eye protection, and follow your laboratory's standard safety practices for handling nucleic acid reagents and PCR consumables.

Dispose of reagents, consumables, and used RDG16 Cartridges in accordance with applicable local, regional, and national regulations for laboratory waste. Do not reuse cartridges.

Kit Contents

For details on assay target coverage, refer to the assay-specific Information Sheet.

Each ddPLEX CrystalTag ddPCR Assay is supplied as a ready-to-use, assay-specific mix of ddPCR primers and CrystalTag Probes (orange cap, 10x concentrate). Each assay package also includes a Positive Control (clear cap, 10x) consisting of synthetic DNA (Table 1).

Table 1. Kit contents for ddPLEX CrystalTag ddPCR Assays.

Kit Size	CrystalTag Assay	Positive Control
200 x 5 µL reactions	120 µL x 1	120 µL x 1
1000 x 5 µL reactions	120 µL x 5	120 µL x 1

QX700 CrystalTag Reporters are ordered separately and supplied as a 3-plex or 7-plex at a 40x stock concentration (Table 2).

Table 2. Kit contents for QX700 CrystalTag Reporters.

Kit Size	CrystalTag Reporter
200 x 5 µL reactions	30 µL x 1
1000 x 5 µL reactions	30 µL x 5

Reagents and Equipment

Additional reagents may be required for certain assays, always refer to assay-specific information sheet.

The following materials are required but not provided:

- For 1-3 targets, QX700 CrystalTag Reporters 3 (#12027210, #12027271)
- For 4 targets and above, QX700 CrystalTag Reporters 7 (#12027244, #12027265)
- For DNA applications, QX700 ddPCR Supermix for Probes (#12025352, #12025428, #12025522).
- For RNA applications, QX700 ddPCR Supermix for Probes (#12025352, #12025428, #12025522)
- QX700 Droplet Digital PCR Systems (E, S, and HT)
- RDG16 Cartridges (#12025252).
- Standard consumables and equipment for reaction-mix preparation include PCR-compliant reaction tubes, a microcentrifuge (~700 × g), a vortex mixer, micropipettes and tips, and nuclease-free water.

Protocol

Step 1. Download technical files

1. From the ddPLEX CrystalTag ddPCR Assay product page, download the following files:
   - **Assay Information Sheet,** containing assay specific information including target coverage, assay PCR and scanning conditions, required reagents, reaction setup volumes, and analysis instructions
   - **ddPCR Protocol file** (nioprotocol), including thermal and scanning conditions
   - **Assay Configuration file** (nioassay), including population definitions and compensation matrix. Do not remove the nca and ncm files from the nioassay file.
2. Transfer the nioprotocol and nioassay files into the QX700 ddPCR System. Both files are required for every run; transfer them together.
3. Ensure all necessary reagents listed in the Assay Information Sheet are available in your laboratory. QX700 CrystalTag Reporters should match the multiplex level of the assay.

Step 2. ddPCR Recommendations

4. Prepare samples and experimental planning accounting for the following recommendations:

- DNA Amount to Include in ddPCR Reaction Mix: as a recommendation for optimal performance, do not exceed a chamber concentration (DNA concentration in the reaction mix) of 1,000 copies/µL. For example, this corresponds to approximately 3.3 ng/µL of human genomic DNA. Use the genome size of the species being analyzed, together with available online calculators,

to convert between copies and mass of DNA

- DNA digestion: for DNA samples with ≥10 kb average length (e.g., genomic DNA), digest with a restriction enzyme that does not cut within the target amplicon to improve template accessibility and reduce sample viscosity. Digestion is not required for already-fragmented DNA such as cfDNA or FFPE-derived DNA. Care must be taken to use restriction enzymes that do not cut within the amplified sequence or the CrystalTag Probes. For a list of restriction enzymes compatible with a given ddPLEX CrystalTag Assay, contact our Technical Support team
- Replicates and controls: when running technical replicates, assemble a common reaction mix (enough for ~1.5x the number of wells you plan to run) with all required components, and always include the supplied positive control

Step 3. Prepare and load the reaction mix

5. Completely thaw all reagents:
   - ddPLEX CrystalTag ddPCR Assay
   - Positive Control
   - QX700 CrystalTag Reporters
   - Chosen supermix
6. Vortex all tubes 10 seconds at maximum speed and spin briefly to collect material at the bottom of each tube.
7. Prepare the reaction mix according to the applicable Assay Information Sheet, then vortex and spin it down.
8. Dispense the appropriate volume of reaction mix into standard PCR tubes or plates. The mix contains all components except the template DNA.
9. Add the template DNA (samples, positive control, or negative control) to each tube or well according to the test plan. To limit contamination, close each tube after adding template and prepare the positive control last.
10. Vortex the tubes thoroughly, then briefly centrifuge to collect the contents at the bottom of the tubes.
11. Load 5 µL of reaction mix per chamber into the RDG16 cartridge.
12. Perform the ddPCR reaction on the QX700 ddPCR System.

Step 4. Run the ddPCR reaction on QX700 ddPCR System

13. Open the QX700 ddPCR System Control Software.
14. In the “DIGITAL EXPERIMENTS” section, select “New” and click on “Start from a blank experiment”
15. Under the “Files” box, under Protocol tab, click on “Add file” and browse the previously saved nioprotocol
16. Drag and drop the nioprotocol file to the “Protocol” field underneath each cartridge layout according to desired experimental setup
17. Under the “Files” box, under Assay tab, click on “Add file” and browse the previously saved nioassay
18. Drag and drop the .nioassay of the cartridge plate layout according to desired experimental setup. The nioassay file embeds the compensation matrix and analysis file
19. Modify the sample name for each chamber according to experimental plan
20. Select “Save As” to register the newly created Digital Experiment (nioexperiment) to be used with the desired experimental setup with the ddPLEX CrystalTag ddPCR Assay. *The saved nioexperiment can be used as a template for future experiments with the same experimental setup.*
21. Select “Plan & Start” in the menu “RUNS”, browse and load the nioexperiment file saved in previous step.
22. Load the RDG16 Cartridges into the QX700 ddPCR System.
23. Select the “Start Run” button.
24. Select “Status” in the menu “RUNS” to verify that the RDG48 enters the “Thermocycling” step process if no other previously inserted plate is waiting in the instrument. Otherwise, the freshly inserted RDG48 will be queued. The RDG48 will continue to be scanned once the thermocycling step is complete.
25. At the end of run, copy the results file (niodata) on a USB flash drive.
26. To recover the RDG16 Cartridges, go to “Status” in the menu “RUNS” and select the QX700 Run to unload. Click on “Unload Chip plate” on the right side of the user interface.

Data Acquisition and Analysis

Refer to Assay Information Sheet for detailed instructions and representative data illustrations.

27. Open the experiment results in the QX700 ddPCR System Analysis Software
28. On the 1D plot for each channel, set thresholds to separate positive and negative droplets. For some assays, analysis is performed with polygons on 2D plots; refer to the assay-specific Information Sheet for threshold or polygon settings.
29. Review the concentration of each target under “View Results”.

Quality Control

Each ddPLEX CrystalTag ddPCR Assay is tested on synthetic DNA via functional ddPCR testing prior to release.

QX700 CrystalTag Reporters are manufactured under stringent specifications to ensure lot-to-lot consistency and tested via functional ddPCR testing prior to release.

Visit **www.bio-rad.com/ddplex-crystaltag-ddpcr-assays** for more information.

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