Crystal Digital PCR® Assay
for use with Crystal Universal Reporters

Instructions for Use

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

Product Name

Crystal Digital PCR® Assay

Number of reactions: 200 and 1000 reactions (Ruby Chip)

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Description

A Crystal Digital PCR® Assay allows for DNA quantification of specific targets using Stilla®'s exclusive detection chemistry featuring Crystal Flex Probes and Crystal Universal Reporters in digital PCR. Crystal Flex Probes are non-fluorescent oligonucleotides specific to the target of interest. They contain a non-target specific sequence, a tag oligonucleotide that triggers the fluorescence of their complementary Crystal Universal Reporter when released during PCR.

Stilla®'s unique Color Combination high-plexing assay design technology makes detecting more than seven targets individually accessible. Color Combination is used in Crystal Digital PCR® Assays when higher order multiplexing is required to detect more targets than fluorescent channels available in a single dPCR reaction.

A wide variety of catalog assays is available for applications such as point mutation detection and quantification, titration and genome integrity assessment for cell and gene therapy, and microbial species screening. For the list of available assays, visit the website of Stilla Technologies. Crystal Flex Probe detection chemistry is also available for custom assay design requests, from modifications of catalog assays to fully tailored panel design.

Resources

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 Crystal Digital PCR® Assays.

Technical resources for Stilla Technologies Crystal Digital PCR® Assays can be accessed at:

https://www.stillatechnologies.com/digital-pcr/naica-system-support/technical-resources/

Before starting, consult the Instructions for Use of the naica® PCR MIX and Ruby Chip and the User manual of the Nio™, Nio™+, or naica® system for more information on how to use these products.

Intended Use

A Crystal Digital PCR® Assay is provided in a ready-to-use, assay-specific, PCR primers and Crystal Flex Probes mix, optimized for use with the naica® PCR MIX 10X (R10106) and the Ruby Chip (C16011), to be ordered separately.

The assay is available in 200 and 1000 reaction formats. Crystal Digital PCR® Assays are wet lab validated on synthetic DNA. Sample matrix may have an impact on the assay performance. Individual sample-type and extraction method compatibilities for digital PCR applications may require a dedicated assay validation by the end user. Positive controls are available and included with the assay.

They are intended to be used with Crystal Universal Reporters, to be ordered separately.

Crystal Digital PCR® Assays are intended for use with all configurations of the Nio™ as well as for the 6-color naica® system and 3-color naica® system, provided that the chosen Crystal Universal Reporters match the detection channels of the instrument (Table 1).

Crystal Digital PCR® Assays may require additional user optimization and validation.

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

Reporter	Reporter color	Compatible channels		
		Nio™, Nio™+	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*

Composition

The Crystal Digital PCR® Assay contains the components listed in Table 2, which are available in 200 or 1000 Ruby Chip reaction formats.

Table 2. Composition of the Crystal Digital PCR® Assay

Component Name	Cap Color	Initial Concentration	Volume 200 / 1000 rxn	Description
Crystal Digital PCR® Assay	Orange ●	10X	120 / 600 µL	Ready to use primer & probe solution based on Crystal Flex Probe technology.
Positive Control	Clear ○	10X	120 µL	Synthetic DNA pool

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

Material Required but Not Provided

- Crystal Universal Reporters. For Crystal Digital PCR® assays with multiplex level up to three colors, only Crystal Universal Reporter 3 (Tube A) is required. For higher multiplex levels, Crystal Universal Reporter 7 (Tube A and Tube B) are required.

Table 3. Crystal Universal Reporters product offering for Crystal Digital PCR® Assays

Product Reference	Product Name	Components *Cap Color*	Initial Concentration	Volume 200 / 1000 rxn	Description
R41401 R41402	Crystal Universal Reporter 3	Tube A: *Yellow*	40X	30 / 150 µL	Ready to use reporter solution for detection in the blue, green, and red channels of the Prism3, Prism6, Nio™, and Nio™+
R42401 R42402	Crystal Universal Reporter 7	Tube A: *Yellow*	40X	30 / 150 µL	Ready to use reporter solution for detection in the blue, green, and red channels of the Prism3, Prism6, Nio™, and Nio™+
		Tube B: *Brown*	40X	30 / 150 µL	Ready to use reporter solution for detection in the teal, yellow, infra-red, and long-shift channels of the Prism6, Nio™, Nio™+ (long-shift only available on Nio™ and Nio™+)

- Nio™ or Nio™+ or 6-color naica® system or 3-color naica® system
- Ruby Chip consumables (Reference C16011)
- naica® PCR MIX 10x (Reference R10106)
- Standard consumables and equipment for PCR reaction mix preparation:
  - PCR-compliant reaction tubes
  - PCR-compliant 8-well strip tubes (*optional*)
  - Centrifuge for microcentrifuge tubes (~700xg)
  - Laboratory mixer - Vortex
  - Micropipettes
  - Multi-channel micropipettes (*optional*)
  - Micropipette tips
- Nuclease-Free Water
- Antistatic wetted wipes (ACL Staticide®, Reference: SW12)

  ➔ The specific product can be ordered from Stilla® as a spare part (Part number H10000.472) or directly from the supplier Digi-Key using the reference ST1059-ND. SW12 ACL Staticide Inc | Anti-Static, ESD, Clean Room Products | DigiKey
- Precision Wipes (Kimtech™ Science, Reference: 7552, 1 ply, 213x114 mm) can be ordered from standard laboratory suppliers

Storage

Crystal Digital PCR® Assays are shipped on dry ice. Immediately upon reception, inspect package integrity and ensure the correct storage of the Crystal Digital PCR® Assay at the indicated storage temperatures. In case of doubt on the integrity or correct storage of the assay upon reception, do not use the assay and contact Technical Support.

- All reagents provided in the Crystal Digital PCR® Assay must be stored at -20°C ± 5°C in the original tubes until the expiration date indicated on the respective packaging. 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 protected from light sources.
-  Store tubes at all times in an upright position.
- General consideration for reagent storage: all tube caps should be well-closed before stocking.

Under these conditions, the Crystal dPCR® Assay is stable 12 months after reception.

Before Starting

For optimal performance, it is recommended to not exceed a chamber concentration (DNA concentration in the reaction mix) of 1000 copies/µL. For example, the corresponding human DNA to a DNA concentration in the reaction mix of 3.3 ng/µL. Correspondence between copies and DNA can be determined using calculators accessible on the internet. The necessary information is the genome size of the species being analyzed.

Protocol

1. Operate the Crystal Digital PCR® Assay at a temperature ranging from +20°C to +25°C.
2. Ensure to completely thaw all the reagents below prior to reaction mix preparation:
    - Crystal Digital PCR® Assay
    - Crystal Universal Reporters
    - Positive Control (**recommended to include in test plan to optimize thresholding**)
    - naica® PCR MIX 10X
3. Vortex all tubes (ensure to vortex the naica® PCR MIX Buffer A for at least 10 seconds), 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 **Information Sheet** of each assay.
5. Vortex and spin down the PCR reaction mix.
6. Dispense appropriate volumes of the reaction mix, which contains all components except the template DNA, into standard PCR tubes or plates.
7. Add the template DNA (samples, positive control, or negative control) to each tube or well according to the test plan. It is recommended to close each tube after DNA template addition to limit the risk of contamination. The positive control is recommended to be prepared last, to avoid contamination of the samples.
8. Vortex the tubes thoroughly to mix all reagents and briefly centrifuge to collect the content at the bottom of the tubes.
9. Load 5 µL of reaction mix per chamber in the Ruby Chip consumable (please refer to the Ruby Chip IFU for instructions to load a Ruby Chip).
10. Perform the digital PCR. See details below.

On the Nio™ and Nio™+

Download the Information Sheet specific for the desired Crystal Digital PCR® Assay for details on its thermocycling protocol and scanning parameters.

For instructions on how to run a Crystal Digital PCR® experiment with the Nio™ or Nio™+ and how to analyze results in Nio™ Analyzer, please refer to the respective user manual.

For each Crystal Digital PCR® Assay, the Nio™ protocol and Nio™ Assay files are available for download from the Technical Resource section of the website.

1. Open the Nio™ Reader software.
2. In the “DIGITAL EXPERIMENTS” section, Select “New” and click on “Start from a blank experiment”
3. Under the files box:
    a. In the Protocol tab, click on “Add file” and browse the .nioprotocol
    b. In the Assay tab, click on “Add file” and browse the .nioassay
4. Drag and drop the .nioprotocol file to the “Protocol” field underneath each chip layout according to desired experimental setup.
5. Drag and drop the .nioassay of the chip plate layout according to desired experimental setup. The Nio™ Assay file embeds the compensation matrix and analysis file.
6. Modify the sample name for each chamber according to experimental plan.
7. Select “Save As” to register the .nioexperiment to be used with the desired experimental setup with the Crystal Digital PCR® Assay. The saved .nioexperiment can be used as a template for future experiments with the same experimental setup.
8. Select “Plan & Start” in the menu “RUNS”, browse and load the .nioexperiment file saved in previous step.
9. Load the Chip Plate of the Ruby Chip consumables into the Nio™.
10. Select the “Start Run” button.
11. Select “Status” in the menu “RUNS” to verify that the Chip Plate enters the “Thermocycling” step process if no other previously inserted Chip Plate is waiting in the instrument. Otherwise, the freshly inserted Chip Plate will be queued. The Chip Plate will continue to be scanned once the thermocycling step is complete. Once a chamber is scanned, a quality flag appears in the chamber rectangle (refer to Nio™ Reader software User Manual for detailed instructions for checking image quality).
12. At the end of run, copy the results file .niodata on a USB flash drive.
13. To recover the chips, go to “Status” in the menu “RUNS” and select the “Nio Run” to unload. Click on “Unload Chip plate” on the right side of the user interface.
    ***Note**: Ruby Chip consumables may be rescanned if necessary.*

On the naica® system

Download the Information Sheet specific for the desired Crystal Digital PCR® Assay for details on its thermocycling protocol and scanning parameters.

For instructions on how to run a Crystal Digital PCR® experiment with the Geode and the Prism3 or Prism6, please refer to the respective user manuals. Please refer to the Crystal Reader software User Manual for instructions on how to acquire images with the Prism3 or Prism6 instrument.

For each Crystal Digital PCR® Assay, the PCR program and scanning template files are available for download from the Technical Resource section of the website. The experiment file already embeds the scan settings, as well as the compensation matrix and the analysis files.

1. Place the Ruby Chip consumables 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. Ensure the Prism3 and Prism6 instrument is turned on.
4. Open the Geode lid and transfer the Ruby Chips to the Prism3 or Prism6 instrument.
5. Launch the Crystal Reader software application by double-clicking on the “Crystal reader” icon on the monitor.
6. Click on the “NEW EXPERIMENT” button to create a new experiment with the Ruby Chip.
7. Select the Scanning Template for the Crystal Digital PCR® Assay.
8. A prompt appears, select “Use template chamber details.”
9. Name your experiment (Fill in the “New Experiment” case). It is recommended to enter the experiment name by indicating at least the experiment type as a prefix. Note that date (e.g., YYMMDD) and chip IDs (e.g., XXXXXXXX-XXXXXXXX-XXXXXXXXX) can also be indicated in the experiment name as good practice for file searchability.
10. Enter the Chip ID (either manually or with an external barcode reader).
    In the “Chamber Details” section, modify the sample name for each sample.
11. Click on “Open tray”, place the Ruby Chip consumables in the Prism3 or Prism6 instrument and click on “Close the tray”.
12. Click on “Save” and save the “.ncx” file in the desired folder.
13. Click on “Scan”.
14. After the scan, dispose the Ruby Chip consumables as per recommendations.
    ***Note**: Ruby Chip consumables may be rescanned if necessary.*

Analysis of Results

Open the experiment results in the respective analysis software and set thresholds for separating positive and negative populations on the 1D plots for each channel. For some assays, the analysis is performed with polygons in 2D plots. Refer to the Information Sheet of the specific Crystal Digital PCR® Assay for further instructions on threshold or polygon settings.

Concentration of each target can be found in the “View Results” tab.
Example data is provided in the Information Sheet specific for the desired Crystal Digital PCR® Assay.

Precautions and Warnings

The Crystal Digital PCR® Assay is not classified as dangerous according to Regulation (EC) No. 1272/2008 [CLP].

Appropriate personal protection equipment for handling this product, including lab coat, disposable gloves, and goggles, is required.

Wear additional personal protection equipment when needed. Wash hands before breaks and after work. Remove contaminated, saturated clothing.

In case of exposure:

General information: when in doubt or if symptoms are observed, get medical advice.
Following inhalation: no special measures are necessary. Provide fresh air.
Following skin contact: wash with soap and water.
Following eye contact: in case of eye irritation consult an ophthalmologist. Rinse immediately, carefully, and thoroughly with eyebath or water.
Following ingestion: if swallowed: rinse mouth. Do NOT induce vomiting.
Self-protection of the first aider: no special measures are necessary.

Disposal Considerations

Waste can be considered as a biohazardous waste and must be disposed of according to applicable domestic legislation.
For recycling of the packaging, please consult local or national regulations.

Technical Support Contact Information

Online Technical Support is available at: https://www.stillatechnologies.com/technical-support/

For technical questions or any issue regarding Crystal Digital PCR® Assays, contact us:

Monday to Friday, 9:30 AM – 6:30 PM, Central European Time (CET).
Closed on French bank holidays.
Phone: (+33) 09 82 27 47 47
Email: support@stilla.fr

Patents: https://www.stillatechnologies.com/patents/

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Stilla Technologies
F-94800 Villejuif, FRANCE