naica® IQ/OQ Kit [Nio™ Digital PCR]

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

Product Name

naica® IQ/OQ Kit (Ref. R30001)

Number of reactions: 96 reactions (Ruby Chip)

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Resources

Documents referenced in these Instructions for Use (IFU) are available here:

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

Intended Use

The naica® IQ/OQ Kit is a ready-to-use kit intended to be used for the operational qualification (OQ) of all of the configurations of the Nio™ digital PCR instrument (Nio™ E, Nio, or Nio™+) by qualified laboratory personnel trained to perform Crystal Digital PCR®. Note that in the following instructions, the instrument is referred to as Nio™ and it applies to any of the instrument's configuration (Nio™ E, Nio, or Nio™+) unless otherwise specifically stated.

The naica® IQ/OQ Kit is used with Ruby Chip consumables for IQ/OQ of the Nio™. For detailed instructions about Ruby Chip use, refer to the respective IFU. The 6-color assay of the naica® IQ/OQ Kit is intended to be used with the Nio™.

The installation qualification (IQ) and initial OQ of the Nio™ are only performed by qualified Stilla® personnel or representative designated by Stilla Technologies.

After successful IQ/OQ of the Nio™ by Stilla® personnel, routine OQ procedures of the Nio™ with the naica® IQ/OQ Kit allows for closer monitoring of equipment compliance to operating specifications.

The naica® IQ/OQ Kit is intended to be used only with the sample material included in the kit. No external samples can be used with the naica® IQ/OQ Kit.

The naica® IQ/OQ Kit is not intended for any performance qualification (PQ) of the Nio™.

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Composition

The naica® IQ/OQ Kit is packaged in a sealed box containing a total of six tubes:

Table 1. Components of the naica® IQ/OQ Kit

Name	Cap Color	Initial Concentration	Volume	Description
Buffer A	Blue	10X	70 µL	naica® multiplex PCR MIX Buffer A
Buffer B	Red	100%	30 µL	naica® multiplex PCR MIX Buffer B
Buffer C	Yellow	20X	35 µL	Ready to use primers and TaqMan™ probes with fluorophores FAM, ROX, Cy5
Buffer D	Brown	20X	35 µL	Ready to use primers and TaqMan™ probes with fluorophores YY, ATTO550, Cy5.5
Positive Control*	Purple	25X	300 µL	Synthetic DNA template used as positive controls
Nuclease-Free Water	Clear	N/A	560 µL	Nuclease-free water for volume adjustment

*Positive Control is provided in excess.

Required software files and programs for the naica® IQ/OQ Kit (.nioexperiment file and Excel Analysis Template file) can be downloaded from the technical resource page of the Stilla website.

Materials Required but Not Provided

- 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
- Ruby Chip consumables (Reference C16011)
- 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

Immediately upon reception, inspect package integrity and ensure the correct storage of the naica® IQ/OQ Kit at the indicated storage temperatures. In case of doubt on the integrity or correct storage of the naica® IQ/OQ Kit upon reception, do not use the kit and contact Technical Support.

- All components provided in the naica® IQ/OQ Kit must be stored at -20°C ± 5°C in original tubes until the expiration date indicated on the respective packaging. Do not aliquot in alternative tubes.
-  Store tubes protected from light sources. It is recommended to store all tubes in the provided cardboard box.
-  Store tubes at all times in an upright position. It is recommended to store all tubes in the provided cardboard box.
- The naica® IQ/OQ Kit can be thawed up to 6 times without observable deviations in performance and shelf-life.
- General consideration for reagent storage: all tube caps should be well-closed before stocking.

Under these conditions, the naica® IQ/OQ Kit is stable until the expiration date indicated on the external packaging label.

Conditions for Use

- Operate the naica® IQ/OQ Kit at a temperature ranging from +20°C to +25°C.
- Ensure to thaw completely each naica® IQ/OQ Kit reagent prior to reaction mix preparation for up to 30 minutes.
-  Before each use, vortex each component thoroughly at maximum speed for 10 seconds and briefly centrifuge to collect the liquid at the bottom of the tube.
- Discard all naica® IQ/OQ Kit reagent components as soon as one reagent component is empty.
- Discard all naica® IQ/OQ Kit reagent components as soon as one reagent component is expired. The expiration date of the limiting component is indicated on the external packaging.
- Never combine naica® IQ/OQ Kit reagent components from different naica® IQ/OQ Kit boxes.

Assay Protocol

The following paragraphs describe how to prepare the naica® IQ/OQ Kit reaction mix, perform the Crystal Digital PCR® reaction and acquire the images on Nio™ Digital PCR, and analyze results with the naica® IQ/OQ Kit and the Ruby Chip.

Step 1. Reaction Preparation

Estimated reaction preparation time for the OQ run: 40 minutes.

The Nio™ E/Nio™ OQ run consists of one run (Run 1) of three Ruby Chip consumables with all chambers filled with positive controls. This run qualifies the single thermocycler of the Nio™ E and Nio™.

The Nio™+ OQ run consists of two runs (Run 1 + Run 2) of three Ruby Chip consumables each (total of six Ruby Chip) with all chambers filled with positive controls. Each run qualifies one of two thermocyclers of the Nio™+.

1. Thaw completely each naica® IQ/OQ Kit reagent at room temperature (+20°C to +25°C) for up to 30 minutes, vortex all tubes for 10 seconds at maximum speed, and spin briefly in a centrifuge to collect the contents at the bottom of each tube.
2. Assemble the reagents as shown in **Table 2**. The reaction mix volumes displayed in **Table 2** allow for sufficient volume for the Nio™ E/Nio™ OQ run (Run 1) and Nio™+ OQ run (Run 1 + Run 2), which are composed by 48 Ruby Chip chamber and 96 Ruby Chip chambers, respectively.

Table 2. Reaction mix setup for Nio™ Digital PCR OQ run with the Ruby Chip

Component Name	Nio™ E/Nio™ OQ run (Run 1) Volume (µL) for 48 reactions	Nio™+ OQ run (Run 1 + Run 2) Volume (µL) for 96 reactions
Buffer A ●	31.7	63.4
Buffer B ●	12.7	25.3
Buffer C ●	15.8	31.7
Buffer D ●	15.8	31.7
Positive Control ●	12.7	25.3
Nuclease-Free Water ○	228.1	456.2
Total volume	*316.8*	*633.6*

3. After assembly, vortex the reaction mix tube for 10 seconds at maximum speed to mix all reagents and briefly centrifuge to collect the contents at the bottom of the tube.
4. (*Optional*) For the use of multi-channel micropipettes, transfer 75 µL of the reaction mix into each of the wells of the 8-well strip tubes.
5. Refer to the Ruby Chip IFU for instructions on the anti-static protocol and general loading procedure of the Ruby Chip.
6. Load 5 µL of the reaction mix into each chamber of the Ruby Chip consumables, in total 48 or 96 chambers for the Nio™ E/Nio™ and Nio™+, respectively. It is recommended to load the Ruby Chip consumables immediately before starting the run on Nio™ Digital PCR.

Step 2. Partition, Amplification, and Image Acquisition

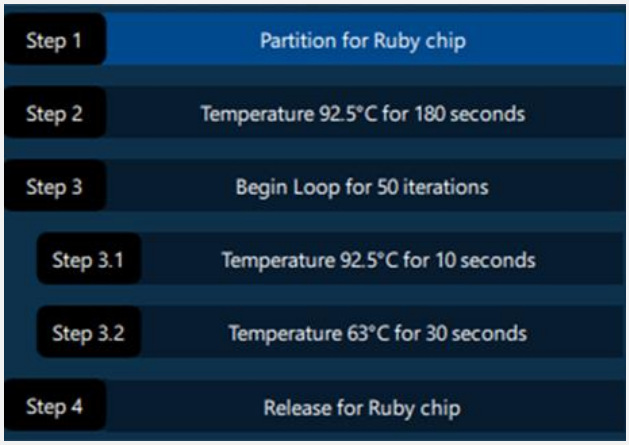
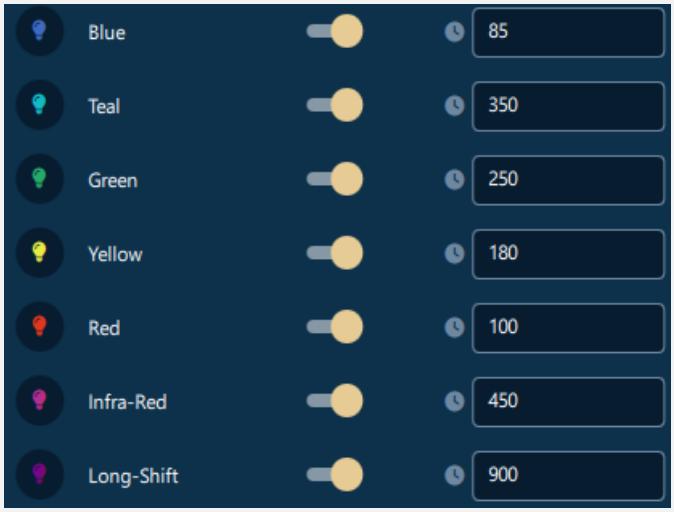
Download the **.nioexperiment** from the technical resources section of Stilla® website on a USB flash drive to import it to the Nio™. Please refer to Nio™ Digital PCR User Manual for details concerning Nio™ use.

Estimated duration of partitioning, amplification, image acquisition for Nio™E/Nio™ OQ (Run 1): 2h 45min.

Estimated duration of partitioning, amplification, image acquisition for Nio™+ OQ (Run 1 + Run 2): 3h 15min.

The following .nioexperiment for naica® IQ/OQ Kit with the Ruby Chip is available:

Table 3: Thermocycling and Reading programs parameters

1. Thermocycling	2. Reading
 **Temperature ramp rate of 1°C/sec.**	

1. Open Nio™ Reader software.
2. Select “Plan & Start” in the menu “RUNS”, browse and load the dedicated .nioexperiment file.
3. Load the Chip Plate with three Ruby Chip consumables into the Nio™.
4. Select the “Start Run” button to start Run 1.
5. Select “Status” in the menu “RUNS” to verify that the first Chip Plate enters the “Thermocycling” step process.

***Nio™+ only*:**

6. Select “Plan & Start” in the menu “RUNS”, load the same .nioexperiment as in previous steps to launch the second run.
7. Load the Chip Plate of the second set of three Ruby Chip consumables into the Nio™+.
8. Select the “Start Run” button to start Run 2.
9. Select “Status” in the menu “RUNS” to verify that the second Chip Plate enters the “Thermocycling” step process.

10. At the end of OQ run, copy the results file **.niodata** of Run 1 (and Run 2 for Nio™+) on a flash drive.
11. 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.*

Step 3. Data Analysis with Nio™ Analyzer

Download the **Analysis Template** of naica® IQ/OQ Kit corresponding to the respective naica® IQ/OQ Kit lot number for the OQ run from the technical resources section of Stilla® website:

“AnalysisTemplate_Nio_RubyChip_naicaIQOQKit_Lot-number.xlsx”

Please refer to Nio™ Analyzer software User Manual for instructions on general use.

1. Open the Run 1 .niodata file generated by the Nio™ Reader software in the Nio™ Analyzer software.
2. Click on “QUALITY CONTROL” and check the Droplet Crystals quality for all chambers in blue channel.
3. Under “ANALYZE DATA”, click on “Explore Crystals” and select the rain in Yellow Channel, increase “Population opacity.”
4. In “Channel Selection”, click on Yellow Channel and screen for droplet inhomogeneities in Droplet Crystals in all chambers.

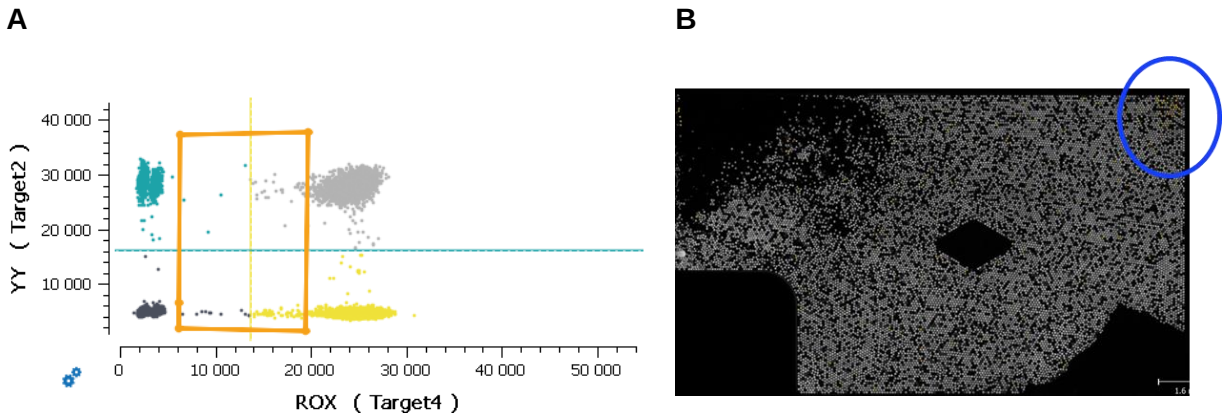


Figure 1. (A) Selection of the Yellow Channel rain zone to (B) screen for thermal inhomogeneity in the Droplet Crystal.

5. In case Droplet Crystal observations are present in any of the chambers (examples of Droplet Crystal observations include air bubbles, dust particles, few analyzable droplets, among others), note the chamber number and refer to **Step 4. Interpretation of Results** for additional instructions.
6. Under “ANALYZE DATA”, click on “Plots & Populations” and then “1D dot plot”. With the selection of all chip chambers, check that the threshold is well-placed for all channels. The threshold must be centered between the mean fluorescence of the positive population and the mean fluorescence of the negative population. If needed, manually adjust the threshold:

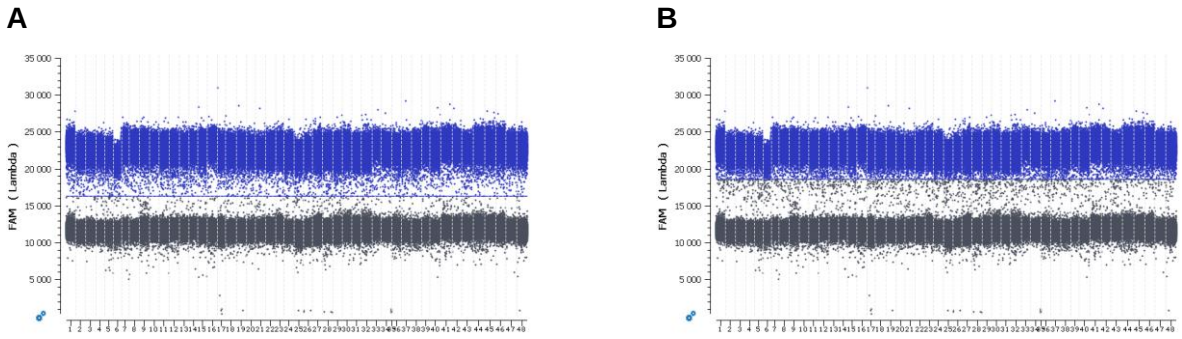


Figure 2. 1D dot plot with (A) a correctly placed threshold and (B) a misplaced threshold

7. Place the threshold of the Long-Shift channel at **15 000 RFU**. For the Long-Shift Channel, as no DNA target is associated to this channel, only the negative population will be visible.

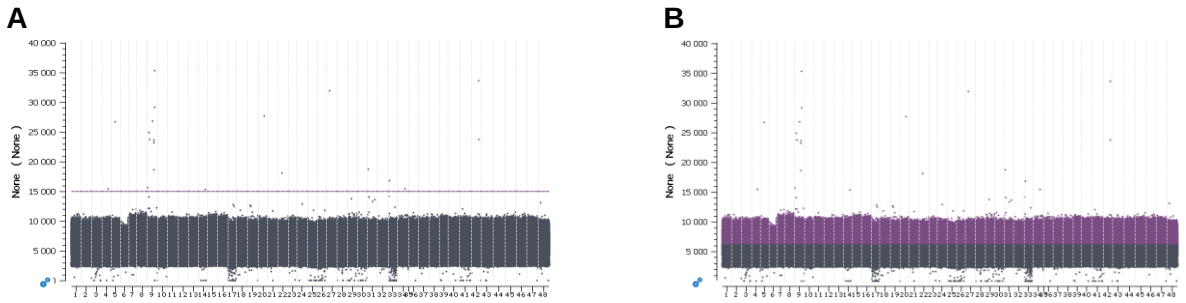


Figure 3. 1D dot plot with (A) a correctly placed threshold and (B) a misplaced threshold for the Long-Shift channel.

8. Go to the “EXPORT” menu and click on the “Browse” button to select the output folder, then click on the “Export” button to export the data (under the same name as the experiment name). A pop-up window will indicate that the data has been successfully exported. A pop-up window will show the Excel table file and Spillover compensation file of the exported data.
9. Go to “FILE > Save” to save the experiment in **.nioresult** format (under the same name as the experiment name).
10. Double-click the exported Excel table to open the file.
11. Open the Analysis Template of the naica® IQ/OQ Kit of the respective kit lot number.
12. Copy the values from the tab “Results” from the exported Excel table to the tab “Results_Run 1” of the Analysis Template.
13. Copy the values from the tab “QC_Advanced” from the exported Excel table to the tab “QC_Advanced_Run 1” of the Analysis Template.
14. Click on the tab “Report_Run 1” of Analysis Template and check for pass/fail.
    Note: Disregard “Results_Run 2”, “QC_Advanced_Run 2” and “Summary” tabs.

    ***Nio™+ only*:**
15. Repeat steps 1-10 with Run 2 .niodata file generated by the Nio™ Reader software and paste the result values into the “Results_Run 2” and “QC_Advanced_Run 2”
16. Save the Analysis Template adding test name and date in the file name.
17. Click on the tab “Report_Run 2” of Analysis Template and check for pass/fail.
18. Click on the tab “Summary” and check for OQ run pass/fail. Fill in run information.

Acceptance Criteria

The intended use of the naica® IQ/OQ Kit is the operational qualification of Nio™ Digital PCR during routine OQ procedures with the Ruby Chip. The correct functioning of the Nio™ is assessed with a verified range of parameters of operational indicators.

The average values from at least 43 Ruby Chip chambers obtained from a single OQ run must be within the accepted range for the OQ to be recorded as conform. The following table lists the OQ acceptance criteria after a OQ run with the Ruby Chip.

Table 4. Acceptance criteria for the IQ/OQ run with the Ruby Chip on Nio™ Digital PCR

Measurement	Target Channel	Accepted Range
Droplet number	N/A	>10 000 per chamber
Average Concentration naica® IQ/OQ Kit, lot dependent	BLUE	Characterized value +/- 15% cp/µL
	TEAL	Characterized value +/- 15% cp/µL
	GREEN	Characterized value +/- 15% cp/µL
	YELLOW*	Characterized value +/- 15% cp/µL
	RED	Characterized value +/- 15% cp/µL
	INFRA-RED	Characterized value +/- 15% cp/µL
Concentration Relative Standard Deviation	BLUE	<7%
	TEAL	<7%
	GREEN	<7%
	YELLOW	<8%
	RED	<8%
	INFRA-RED	<7%
Average Separability Score	BLUE	>7
	TEAL	>14.9
	GREEN	>12.1
	YELLOW	>11.2
	RED	>10.1
	INFRA-RED	>7
Separability Score Relative Standard Deviation	BLUE	<16%
	TEAL	<12%
	GREEN	<14%
	YELLOW	<10%
	RED	<11%
	INFRA-RED	<13%
Fluorescence of the negative population (Mu_neg)	LONG-SHIFT	1200-5000 RFU

*Average concentration of the yellow channel target from at least 43 chambers is not an obligatory pass/fail criterion but rather a criterion for reagent variability monitoring. The Analysis Template will display “warning” instead of “fail” for average concentration of the yellow channel target.

Step 4. Interpretation of Results

1. Confirm that the correct version of the Analysis Template corresponding to the respective naica® IQ/OQ Kit lot number is used for accurate interpretation of results.

2. The OQ run data are validated if:
   - Average total number of droplets of Ruby Chip chamber is within acceptance criterion.
   - Target concentrations averaged from at least 43 chambers are within the acceptance range.
   - Relative standard deviations of the target concentrations are within acceptance criteria.
   - Separability scores averaged from at least 43 chambers are within acceptance criteria.
   - Relative standard deviations of separability scores are within acceptance criteria.
   - Fluorescence of the negative population (Mu_neg) is within acceptance criteria.

3. If all acceptance criteria for Run 1 display "PASS", select "Conform" from the dropdown menu of OQ Decision in the "Report_Run 1" tab.

   ***Nio™+ only*:**
   4. If all acceptance criteria for the Run 2 display "PASS", select "Conform" from the dropdown menu of OQ Decision in the "Report_Run 2" tab.
   5. The Nio™+ OQ is "Conform" only if the Run 1 and Run 2 are "Conform". In this case, the statement "Conform" will appear on the global OQ Decision in the tab "Summary" of the Analysis Template.

6. If at least one of the acceptance criteria displays "FAIL" or Droplet Crystal observations are present in any of the chambers:

   1. Identify within the Analysis Template the individual Ruby Chip chambers creating bias on the interpretation of the OQ result. Cross-check if chambers creating bias match noted chambers containing Droplet Crystal observations.
   2. Open the .nioresults file and click on "QUALITY CONTROL" to visualize the Droplet Crystal images to confirm the presence of Droplet Crystal observations and check for possible thermal inhomogeneity. See the section "*Thermal Inhomogeneity"* below for further instructions.
   3. Refer to the troubleshooting section of the Nio™ Reader software User Manual for troubleshooting steps on Droplet Crystal observations.
   4. After troubleshooting, if no thermal inhomogeneity is observed, the user can exclude up to **five** Ruby Chip chambers per OQ run from the analysis by unselecting the chamber to be excluded (select 0 in the Column "Exclude chambers?"). Note the justification of the exclusion in the dedicated space of the Analysis Template. For better traceability, do not remove the affected chamber from the .nioresult file.
      ***Note**: chambers in position A and H cannot be excluded by pairs within the same chip (i.e. A1-A2 or H1-H2) from the Analysis Template for a Nio™ qualification. In case of Droplet Crystal observations in chambers pairs A1-A2 or H1-H2, reconduct the OQ run. See section "Thermal Inhomogeneity" for more information*.
   5. Reassess tab "Report" of Analysis Template and check for pass/fail.
   6. If all acceptance criteria display "PASS", select from the dropdown menu of OQ Decision, "Conform" and finalize OQ documentation.

7. If at least one of the acceptance criteria display "FAIL" after exclusion of Ruby Chip chamber(s) with Droplet Cystal observations, select "Not Conform" from the dropdown menu of OQ Decision and contact our Technical Support Team.

Note: The average separability scores of the targets detected green and infra-red channels are sensitive to the presence of Droplet Crystal observations, in particular to the presence of fluorescence aggregates (Figure 4). The Analysis Template will display "Check for the presence of Droplet Crystal observations" when average separability scores of these targets are close to the acceptance criteria limit.

To check for the presence of Droplet Crystal observations:

1. Open the **.nioresult** file, click on "QUALITY CONTROL", and select the channel of interest (i.e. green and infra-red channels).
2. Unselect the "Show All" for a closer inspection of the Droplet Crystal quality.
3. If Droplet Crystal observations are not detected, no additional action is required.
4. If Droplet Crystal observations are detected, add a note in the dedicated space of the Analysis Template for traceability purposes.

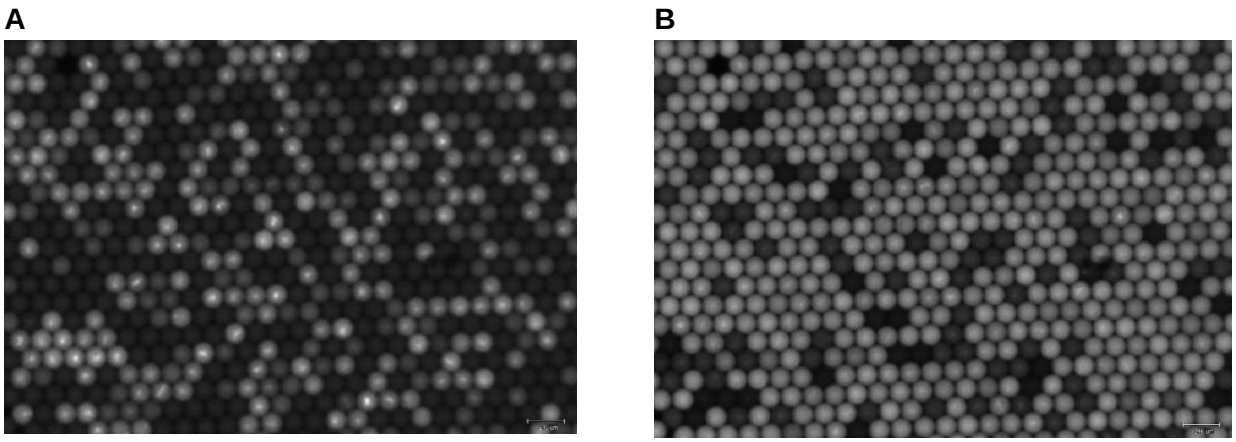


Figure 4. Example of (A) fluorescent aggregates and (B) subtle fluorescent aggregates in the Droplet Crystal

Thermal Inhomogeneity

Thermal inhomogeneity is the absence of amplification or delayed amplification in droplets due to insufficient heating. Insufficient heating can be caused by a deviation from the optimal calibration of the thermoblock peltiers of the Nio™ Digital PCR thermocycler(s). The absence or delayed amplification causes a lower fluorescence in the droplet, which results in a somber patch/zone in the Droplet Crystal image and rain in the 1D dot plot.

Due to the configuration of the Nio™, thermal inhomogeneity frequently occurs in chambers A and H of the Ruby Chip and is frequently located on the top and bottom of the Droplet Crystal, and occasionally on the right edges (column 1 of Ruby Chip) and left edges (column 2 of Ruby Chip). Thus, all chambers should be examined to verify thermal homogeneity of the thermocycler(s) of the Nio™ Digital PCR instrument.

The naica® IQ/OQ Kit was designed to detect thermal inhomogeneity of the thermocycler(s) of the Nio™. Detecting thermal inhomogeneity is part of the OQ protocol of the Nio™:

1. Inspect Droplet Crystal images of the affected chamber(s):

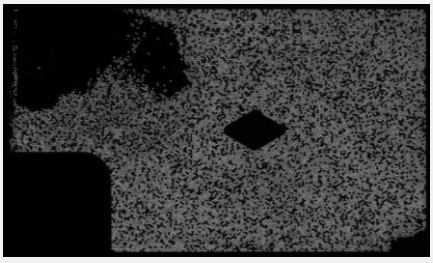
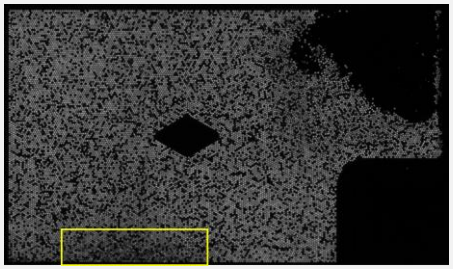
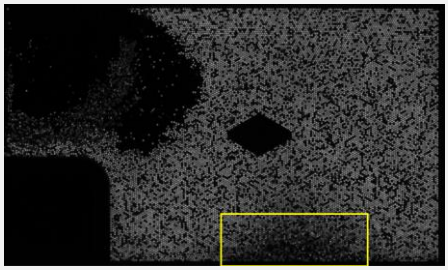
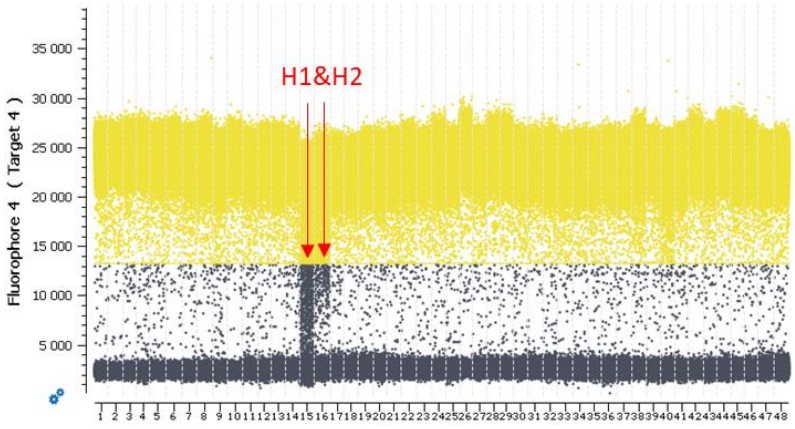
Expected Chamber	Chamber with subtle thermal inhomogeneity	Chamber with thermal inhomogeneity
		

Figure 5. Droplet Crystal images of H chambers of the Ruby Chip: expected vs affected by thermal inhomogeneity.

2. In case of suspected thermal inhomogeneity on the Droplet Crystal, inspect 1D dot plots of the **YELLOW channel.**



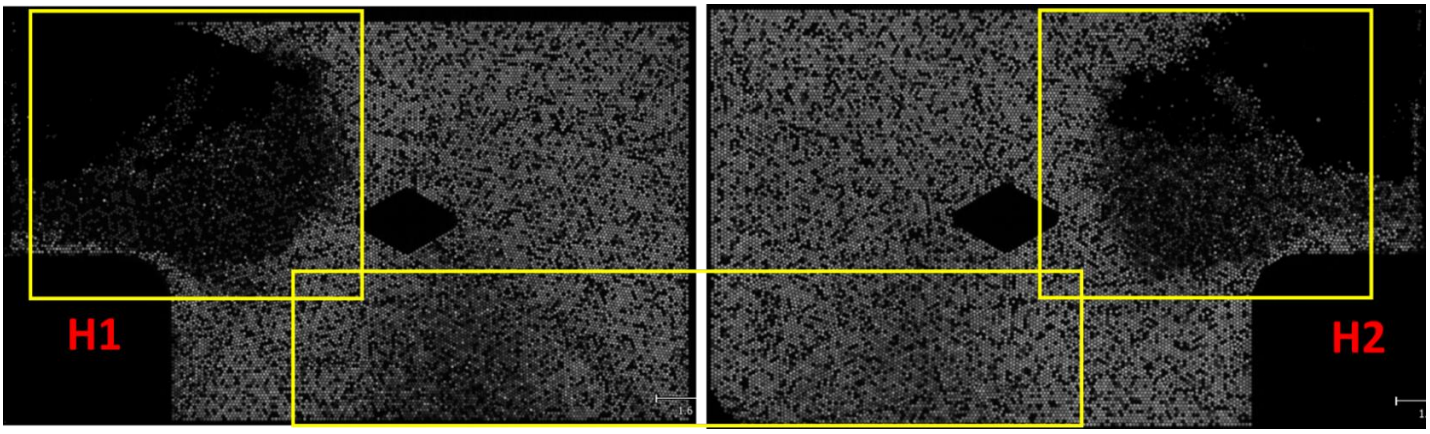


Figure 6. Identifying thermal inhomogeneity in the YELLOW channel.

3. If thermal inhomogeneity is detected in either run, select “Not Conform” from the dropdown menu of OQ Decision and contact our Technical Support Team.

Quality Control

Each batch of naica® IQ/OQ Kit is functionally tested on Nio™ Digital PCR and must perform within the established specifications.
A Certificate of Compliance is available upon request from the Technical Support Department.

Precautions and Warnings

Appropriate personal protection equipment for handling this product, including laboratory coat, disposable gloves, and safety goggles, is required. Wear additional personal protection equipment when needed. Apply Good Laboratory Practices (GLP). 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.

For further information on safety, please refer to the Safety Data Sheet of the naica® IQ/OQ Kit.

Disposal Considerations

Waste can be considered as a biohazardous waste and must be disposed of according to applicable national or local legislation. For recycling of cardboard 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 the naica® IQ/OQ Kit on Nio™ Digital PCR, contact us:

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

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

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