

naica® IQ/OQ Kit

Instructions for Use

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

Product Name

naica® IQ/OQ Kit (Ref. R30001)

Number of reactions: 24 reactions (Sapphire Chip)

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Resources

Documents referenced in this Instructions for Use (IFU) can be accessed 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 the 3-color and 6-color naica® systems by qualified laboratory personnel trained to perform Crystal Digital PCR™ using the naica® system.

The naica® IQ/OQ Kit is used with Sapphire Chip consumables for OQ of the naica® system. For detailed instructions about Sapphire Chip use, refer to the respective Instructions for Use. The 3-color assay of the naica® IQ/OQ Kit is intended to be used only with the 3-color naica® system and the 6-color assay of the naica® IQ/OQ Kit is intended to be used only with the 6-color naica® system.

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

After successful IQ/OQ of the naica® system by Stilla® personnel, routine OQ procedures of the naica® system 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 naica® system.

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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

Component 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

*Buffer D is only required when the naica® IQ/OQ Kit is used on the 6-color naica® system. Discard the Buffer D tube for the use of the naica® IQ/OQ Kit on the 3-color naica® system.

All required software files and programs for the naica® IQ/OQ Kit can be downloaded from [Technical Resources | Stilla Technologies](#):

- Geode Program: "PartitioningProgram_Geode_SapphireChip_naicaIQOQKit_V1"
- Crystal Reader Scanning Template files, including the respective embedded compensation matrix:
 - 3-color naica® system: "ScanningTemplate_Prism3_SapphireChip_naicaIQOQKit_3plex.ncx"
 - 6-color naica® system: "ScanningTemplate_Prism6_SapphireChip_naicaIQOQKit_6plex.ncx"
- Excel Analysis Template files:
 - 3-color naica® system: "AnalysisTemplate_Prism3_SapphireChip_naicaIQOQKit_3plex.xlsx"
 - 6-color naica® system: "AnalysisTemplate_Prism6_SapphireChip_naicaIQOQKit_6plex.xlsx"

Materials Required but Not Provided

- Standard consumables and equipment for PCR reaction mix preparation:
 - PCR-compliant reaction tubes
 - Centrifuge for microcentrifuge tubes (~700xg)
 - Laboratory mixer - Vortex
 - Micropipettes
 - Micropipette tips
- Sapphire Chip consumables (Reference C14012).
- 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 $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ 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 thoroughly (suggested three times of 5-10 seconds each) 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 reagent mixes, perform the Crystal Digital PCR™ reaction, acquire the images, and analyze results with the naica® IQ/OQ Kit. Two protocols are available:

- 3-color naica® system IQ/OQ run protocol
- 6-color naica® system IQ/OQ run protocol

Step 1. Reaction Preparation






3-color naica® system IQ/OQ run reaction preparation

Estimated reaction preparation time for the 3-color naica® system IQ/OQ run: 20 minutes.

The 3-color naica® system IQ/OQ run consists of three Sapphire Chip filled with only positive controls.

1. Thaw completely each naica® IQ/OQ Kit reagent at room temperature (20° to 25°C) for up to 30 minutes, vortex all tubes (ensure to vortex Buffer A for at least 10 seconds), 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 one 3-color naica® system IQ/OQ run including 12 chambers using the Sapphire Chip consumable.

Table 2. Reaction mix setup for one 3-color naica® system IQ/OQ run with the Sapphire Chip

Component Name	Volume (µL)
Buffer A 	32.5
Buffer B 	13.0
Buffer C 	16.3
Positive Control 	13.0
Nuclease-Free Water 	250
Total volume (µL)	325

3. After assembly, vortex the reaction mix tube thoroughly to mix all reagents and briefly centrifuge to collect the contents at the bottom of the tube.
4. Refer to the Sapphire Chip IFU for instructions on the anti-static protocol and general loading procedure of the Sapphire Chip.
5. Load 25 µL of the reaction mix into each chamber of the three Sapphire Chip consumables, in total 12 chambers, within 30 minutes of mix preparation. It is recommended to load the Sapphire Chip consumables directly before starting the run on the Geode instrument.

6-color naica® system IQ/OQ run reaction preparation

Estimated reaction preparation time for the 6-color naica® system IQ/OQ run: 20 minutes.

The 6-color naica® system IQ/OQ run consists of three Sapphire Chip filled with only positive controls.

1. Thaw completely each naica® IQ/OQ Kit reagent at room temperature (20°C to 25°C) for no more than 30 minutes, vortex all tubes (ensure to vortex Buffer A for at least 10 seconds), and spin briefly in a centrifuge to collect the material at the bottom of each tube.
2. Assemble the reagents as shown in **Table 3**. The reaction mix volumes displayed in **Table 3** allow for sufficient volume for one 6-color naica® system IQ/OQ run including 12 chambers using the Sapphire Chip consumable.

Table 3. Reaction mix setup for 6-color naica® system IQ/OQ run with the Sapphire Chip

Component Name		Volume (µL)
Buffer A	●	32.5
Buffer B	●	13.0
Buffer C	●	16.3
Buffer D	●	16.3
Positive Control	●	13.0
Nuclease-Free Water	○	234
Total volume (µL)		325

3. After assembly, vortex the reaction mix tube thoroughly to mix all reagents and briefly centrifuge to collect the contents at the bottom of the tube.
4. Refer to the Sapphire Chip IFU for instructions on the anti-static protocol and general loading procedure of the Sapphire Chip.
5. Load 25 µL of the reaction mix into each chamber of the three Sapphire Chip consumables, in total 12 chambers, within 30 minutes of mix preparation. It is recommended to load the Sapphire Chip consumables directly before starting the run on the Geode instrument.

Step 2. Partition and Amplification

Download the **Geode Program** “PartitioningProgram_Geode_SapphireChip_naicaIQOQKit_V1.js” of naica® IQ/OQ Kit from the technical resources section of the Stilla® website on a USB flash drive to import it to the PCR programs of the Geode. Please refer to Geode User Manual for how to import a PCR program from an USB flash drive and details concerning Geode use.

The Geode Program “PartitioningProgram_Geode_SapphireChip_naicaIQOQKit_V1.js” is used for both the 3-color and 6-color naica® systems.

Estimated duration of the naica® IQ/OQ Kit Geode program: 2 hours and 15 min.

1. Place the Sapphire Chip consumables in the Geode and close the lid.

2. Select the Geode Program from the main menu of the Geode screen and press the “Play”-button to start the program.

Table 4. PCR program “PartitioningProgram_Geode_SapphireChip_naicaIQOQKit_V1”

Step	Temperature (°C)		Duration
Partitioning & Enzyme activation	40°C		Sapphire partitioning
	95 °C		3 min
50 cycles	Denaturation	95 °C	10 sec
	Elongation	62 °C	30 sec
Release	N/A		Sapphire release

3. At the end of the run, the message “PCR completed successfully, touch the screen to continue” appears. Recover the Geode “Live Logs” on a USB flash drive. Open the Geode lid and transfer the Sapphire Chip consumables to the Prism3 or Prism6 instrument.

Note: A Sapphire Chip may be scanned after an overnight PCR run.

Step 3. Image Acquisition with Crystal Reader

Download the latest version of the **Scanning Template** of naica® IQ/OQ Kit from the technical resources section of Stilla® website for the respective naica® system:

- 3-color naica® system: “ScanningTemplate_Prism3_SapphireChip_naicaIQOQKit_3plex.ncx”
- 6-color naica® system: “ScanningTemplate_Prism6_SapphireChip_naicaIQOQKit_6plex.ncx”

3-color naica® system IQ/OQ run image acquisition

Estimated duration of the 3-color naica® IQ/OQ run scan: 15 min for up to 12 Sapphire Chip chambers.

Please refer to Prism3 & Crystal Reader software for the 3-color naica® system User Manual for instructions on how to acquire images with the Prism3 instrument.

1. Ensure the Prism3 instrument is turned on.
2. Launch the Crystal Reader software application by double-clicking on the “Crystal Reader” icon on the monitor.
3. Click on the “NEW EXPERIMENT” button to create a new experiment with the Sapphire Chip.
4. Select the Scanning Template for the 3-color naica® IQ/OQ run:
ScanningTemplate_Prism3_SapphireChip_naicaIQOQKit_3plex.ncx
5. A prompt appears, select “Use default sample details.”

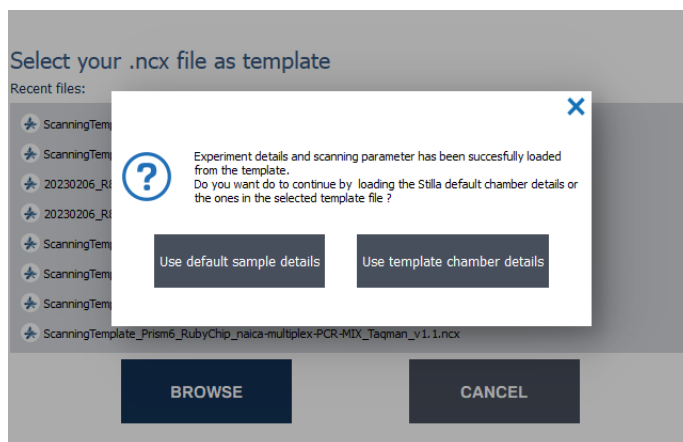


Figure 1. Home menu prompt for sample configuration selection.

6. 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 (i.e., “3-color naica® system IQ/OQ run”). Note that date (e.g., YYMMDD) and Chip IDs (e.g., XXXXXXXX-XXXXXXX-XXXXXXX) can also be indicated in the experiment name as good practice for file searchability.
7. Enter the Chip ID (either manually or with an external barcode reader).
8. Click on “Open tray”, place the Sapphire Chip consumables in the Prism3 instrument and click on “Close the tray”.
9. Click on “Save” and save the “.ncx” file in the desired folder.
10. Click on “Scan”.
11. At the end of the run, store the chips at room temperature away from light on a solid, flat surface. Care should be taken to avoid tilting the chips between movements.
Note: Droplet stability may be impacted by storage environment.

6-color naica® system IQ/OQ run image acquisition

Estimated duration of the 6-color naica® IQ/OQ run scan: 40 min for up to 12 Sapphire Chip chambers.

Please refer to Prism6 & Crystal Reader software for the 6-color naica® system User Manual for instructions on how to acquire images with the Prism6 instrument.

1. Ensure the Prism6 instrument is turned on.
2. Launch the Crystal Reader software application by double-clicking on the “Crystal Reader” icon on the monitor.
3. Click on the “NEW EXPERIMENT” button to create a new experiment with the Sapphire Chip.
4. Select the Scanning Template for the 6-color naica® IQ/OQ run:
ScanningTemplate_Prism6_SapphireChip_naicaIQOQKit_6plex.ncx
5. A prompt appears, select “Use default sample details.”

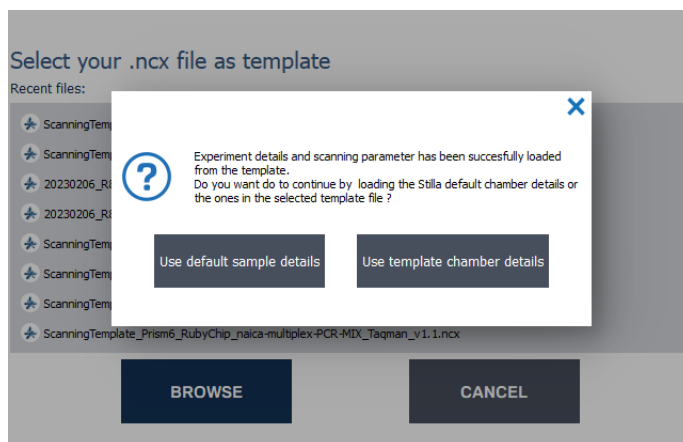


Figure 2. Home menu prompt for sample configuration selection.

6. 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 (i.e., “6-color naica® system IQ/OQ run”). Note that date (e.g., YYMMDD) and Chip IDs (e.g., XXXXXXXX-XXXXXXXX-XXXXXXXX) can also be indicated in the experiment name as good practice for file searchability.
7. Enter the Chip ID (either manually or with an external barcode reader).
8. Click on “Open tray”, place the Sapphire Chip consumables in the Prism6 instrument and click on “Close the tray”.
9. Click on “Save” and save the “.ncx” file in the desired folder.
10. Click on “Scan”.
11. At the end of the run, store the chips at room temperature away from light on a solid, flat surface. Care should be taken to avoid tilting the chips between movements.
Note: Droplet stability may be impacted by storage environment.

Step 4. Data Analysis with Crystal Miner

Download the latest version of the **Analysis Template** of naica® IQ/OQ Kit from the technical resources section of Stilla® website for the respective naica® system:

- 3-color naica® system: “AnalysisTemplate_Prism3_SapphireChip_naicaIQOQKit_3plex.xlsx”
- 6-color naica® system: “AnalysisTemplate_Prism6_SapphireChip_naicaIQOQKit_6plex.xlsx”

Please refer to Crystal Miner software User Manual for instructions on general use.

1. Open the “.ncx” file generated by the Crystal Reader software in the Crystal Miner software after the scanning step by double-clicking the file.
2. Go to “FILE > Save” to save the experiment in “.ncr” format (under the same name as the experiment name).
3. 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.
4. Double-click the exported Excel table to open the file and copy the values from the tab “Results.”

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5. Open the latest version of the Analysis Template of the naica® IQ/OQ Kit and paste these values into the tab “Results.”
6. Save the Analysis Template adding test name and date in the file name.
7. Click on the tab “Report” of Analysis Template and check for pass/fail. Fill in run information.

Acceptance Criteria

The intended use of the naica® IQ/OQ Kit is the operation qualification of the naica® system during routine OQ procedures with Sapphire Chip consumables. The correct functioning of the naica® system is assessed with a verified range of parameters of operational indicators.

The average values from at least 11 Sapphire Chip chambers obtained from a naica® system IQ/OQ run must be within the accepted range for the naica® system OQ to be recorded as conform.

The following tables list the OQ acceptance criteria after a naica® system IQ/OQ run with the Sapphire Chip.

3-color naica® system IQ/OQ run acceptance criteria

Table 5. Acceptance criteria for the 3-color naica® system IQ/OQ run with the Sapphire Chip

Criteria	Target Channel	Accepted Range
Droplets number	N/A	>12 900 per chamber
Average Concentration	BLUE	1700-2300 cp/μL
	GREEN*	5100-6900 cp/μL
	RED	510-690 cp/μL
Concentration Relative Standard Deviation	BLUE	<8%
	GREEN	<9%
	RED	<8%
Average Separability Score	BLUE	>7
	GREEN	>13
	RED	>10
Separability Score Relative Standard Deviation	BLUE	<21%
	GREEN	<31%
	RED	<23%

*Average concentration of the green channel target from at least 11 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 green channel target.

6-color naica® system IQ/OQ run acceptance criteria

Table 6. Acceptance criteria for the 6-color naica® system IQ/OQ run with the Sapphire Chip

Criteria	Target Channel	Accepted Range
Droplet number	N/A	>12 900 per chamber
Average Concentration	BLUE	1700-2300 cp/μL
	TEAL	1700-2300 cp/μL
	GREEN	1700-2300 cp/μL
	YELLOW*	5100-6900 cp/μL
	RED	510-690 cp/μL
	INFRA-RED	510-690 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	>9
	GREEN	>9
	YELLOW	>7.5
	RED	>4.5
	INFRA-RED	>9
Separability Score Relative Standard Deviation	BLUE	<16%
	TEAL	<23%
	GREEN	<18%
	YELLOW	<28%
	RED	<25%
	INFRA-RED	<14%

*Average concentration of the yellow channel target from at least 11 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 5. Interpretation of Results

The naica® system IQ/OQ run data are validated if:

- Total number of droplets for any given Sapphire Chip chamber is within acceptance criterion.
- Target concentrations averaged from at least 11 chambers are within the acceptance criteria range.
- Relative standard deviations of the target concentrations are within acceptance criteria.
- Separability scores averaged from at least 11 chambers are within acceptance criteria.
- Relative standard deviations of separability scores are within acceptance criteria.

If all acceptance criteria display “PASS”, select “Conform” from the dropdown menu of IQ/OQ Decision.

If at least one of the acceptance criteria displays “FAIL”:

1. Open “.ncr” file and click on “Quality Control” to check the Droplet Crystal images for any observation creating bias on the interpretation of the IQ/OQ result and for possible thermal inhomogeneity (see section below for further instructions). Please refer to troubleshooting section of Crystal Reader software User Manual for troubleshooting steps on Droplet Crystal observations.
2. After troubleshooting, if no thermal inhomogeneity is observed, the user can exclude up to **one** Sapphire chamber per IQ/OQ run from the analysis by unselecting the chamber to be excluded in the Analysis Template. Note the justification of the exclusion on the dedicated space of the Analysis Template. For better traceability, do not remove the affected chamber from the .ncr file.
Note: *chamber A1 cannot be excluded from the Analysis Template. In case of Droplet Crystal observations in the chamber A1, reconduct the IQ/OQ run. See section “Thermal Inhomogeneity” for more information.*
3. Reassess tab “Report” of Analysis Template and check for pass/fail.
4. If all acceptance criteria display “PASS”, select from the dropdown menu of IQ/OQ Decision, “Conform”.
5. If at least one of the acceptance criteria display “FAIL” after exclusion of one Sapphire chamber, select “Not Conform” from the dropdown menu of IQ/OQ Decision and contact our Technical Support Team.

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 Geode. 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 Geode, thermal inhomogeneity frequently occurs in chamber A1 of the Sapphire Chip. It can also be observed in other positions thus all chambers should be examined to verify thermal homogeneity of the thermoblock of the Geode.

The naica® IQ/OQ Kit was designed to detect thermal inhomogeneity of the Geode. Detecting thermal inhomogeneity is part of the OQ protocol of the naica® system:

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

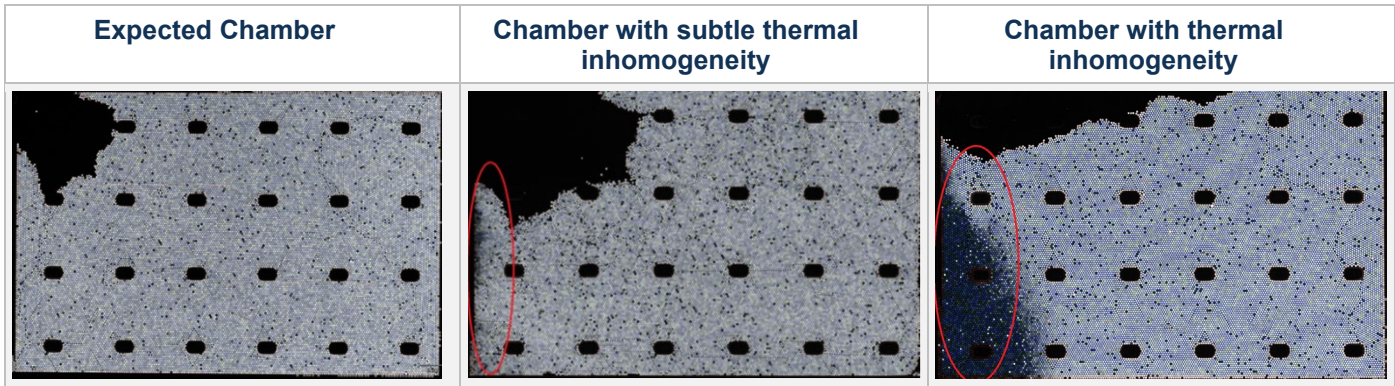


Figure 3. Droplet Crystal images: expected vs affected by thermal inhomogeneity.

2. In case of suspected thermal inhomogeneity on the Droplet Crystal, inspect 1D dot plots:
 - a. For a 3-color run read on a Prism3, inspect the 1D dot plot of the **GREEN channel**.
 - b. For 6-color run read on a Prism6, inspect the 1D dot plot of the **YELLOW channel**.

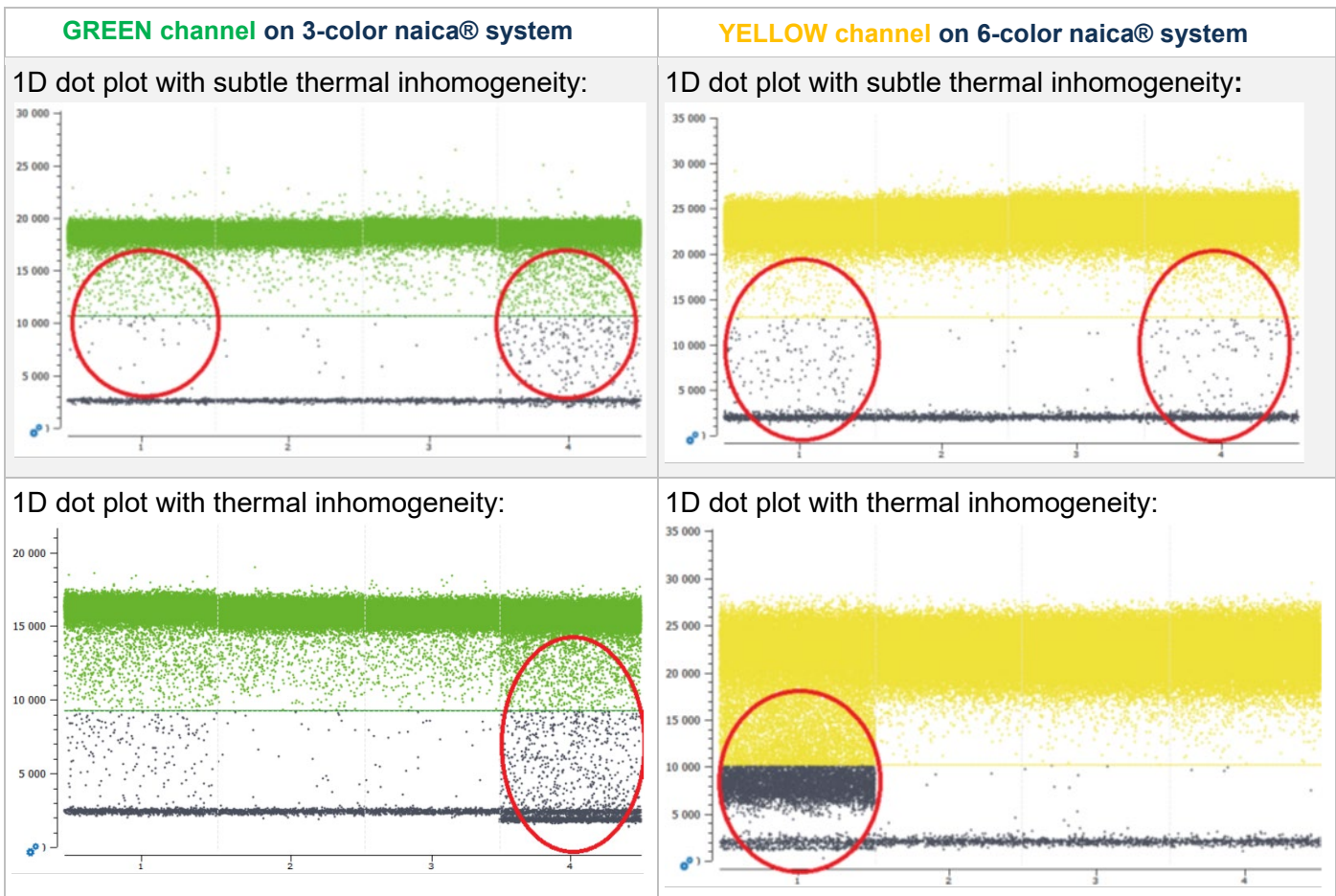


Figure 4. Identifying thermal inhomogeneity on the GREEN (3-color) and YELLOW (6-color) channels.

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

Quality Control

Each batch of naica® IQ/OQ Kit is functionally tested in 3-color and 6-color Crystal Digital PCR™ assays on the 3-color and 6-color naica® systems 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.

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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, contact us:

For customers from Europe, China, and Africa:

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

For customers from America, Asia (excluding China), and Oceania:

Monday to Friday, 8:00 AM – 6:00 PM, Eastern Standard Time (EST).

Closed on American bank holidays.

Phone: 1-833-888-0150 ext.1

Email: support@stilla-inc.com

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

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