



# naica Installation Qualification and Operational Qualification Kit

## Instructions for Use

naica IQ/OQ Kit  
Catalog No. 12025260  
Product Reference No. R30001

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## Section 1: Introduction

### 1.1 Purpose

The naica Installation Qualification and Operational Qualification Kit (naica IQ/OQ Kit) is intended for the installation and operational qualifications of the QX700™ Droplet Digital™ PCR System (QX700 ddPCR™ System).

**Important:** All models of the instrument are for research use only and are **not** intended to be used in diagnostic procedures.

### 1.2 Scope

This document shall be used when qualifying the QX700 ddPCR System, models E, S, and HT. The information and qualification steps herein apply to all models unless specifically stated otherwise.

Note the following:

- The naica IQ/OQ Kit is intended for use only with the sample material included in the Kit . Do not use external samples.
- Only qualified Bio-Rad field service engineers (FSEs), or approved representatives, are authorized to perform IQ and initial OQ testing on the instrument.
- Subsequent customer testing must be performed by ddPCR-qualified and trained laboratory personnel.
- The naica IQ/OQ Kit, with the included six color assay, is ready to use with RDG16 cartridge and RDG48 cartridge plate consumables and read on the seven (7) colors of the QX700 ddPCR System. For information, refer to the Instructions for Use (IFU) DIR 10000171484.
- After successful IQ/OQ tests are executed, the customer can continue monitoring instrument operating specifications and equipment compliance using this protocol. This kit is not intended for performance qualifications of the QX700 ddPCR System.

### 1.3 Protocol Guidelines












The protocol guidelines in this procedure shall be followed. If there is an element that does not apply, it shall be indicated by **N/A** (not applicable). If any section has not been successfully completed, you must indicate in Section 5 the appropriate protocol exceptions and actions taken.

**Note:** This protocol might require the use of a printer to print the specified reports, as requested by the customer.

## Section 2: Requirements

### 2.1 Symbol Lexicon

Table 2.1 Symbol lexicon, naica IQ/OQ Kit label

Symbol	Description
	Use caution; for information, see Section 3, Safety Precautions and Warnings
	Product reference number
	Batch code
	Use-by date
	Manufacturer
	Distributor and catalog number
	Consult instructions for use (paper/electronic)
	Keep away from sunlight
	Do not use if package is damaged; consult instructions for use
	Temperature limit
	This side up

## 2.2 Required Materials Included in Kit

The naica IQ/OQ Kit is packaged in a sealed box and contains the components identified in Table 2.2.

**Table 2.2 Included required materials**

Component	Cap Color	Initial Conc	Volume	Description
Buffer A	Blue	10X	70 µL	naica Multiplex ddPCR Mix Buffer A
Buffer B	Red	100%	30 µL	naica Multiplex ddPCR Mix Buffer B
Buffer C	Yellow	20X	35 µL	Ready-to-use primers and TaqMan probes with fluorophores FAM, ROX, and Cy5
Buffer D	Brown	20X	35 µL	Ready-to-use primers and TaqMan probes with fluorophores YY*, ATTO-550, and 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

\*YY is read in the Teal channel.

\*\*Extra Positive Control sample is provided.

## 2.3 Required Materials Not Included in Kit

You must provide the following items:

- 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
- RDG16 consumables (catalog no. 12025252)
- DigiKey ACL Staticide antistatic wetted wipes (DigiKey catalog no. ST1059-ND SW12)
- Kimtech Kimwipes, 1 ply, 4.4 in x 8.2 in (114 mm x 213 mm); *available from general suppliers.*

## 2.4 Storage Requirements

Immediately upon receipt, inspect package integrity and ensure the correct storage of the naica IQ/OQ Kit at the indicated storage temperatures. If there is any doubt regarding the integrity or correct storage of the naica IQ/OQ Kit upon receipt, **do not** use the kit and contact Bio-Rad Technical Support.

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

- You must store all components provided in the naica IQ/OQ Kit in their original tubes at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  until the expiration date specified on the packaging is reached. **Do not aliquot in alternative tubes.**
- Store tubes in an upright position and ensure they are protected from light sources. Bio-Rad recommends using the cardboard box provided.
- You can thaw the naica IQ/OQ Kit components up to 6x without observable deviations in performance and shelf life.
- Ensure all caps are securely closed before returning them to storage.

## 2.5 Required Conditions for Use

Ensure the following conditions are met before executing the naica IQ/OQ Kit instructions in this IQ/OQ document:

- Operate the naica IQ/OQ Kit at a temperature ranging from  $+20^{\circ}\text{C}$  to  $+25^{\circ}\text{C}$ .
- Thaw each naica IQ/OQ Kit reagent completely (for up to 30 min) before you prepare the reaction mix.
- Before each use, vortex each component thoroughly at max speed for 10 sec, and then 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 is indicated on the external packaging.
- Never combine naica IQ/OQ Kit reagent components from different naica IQ/OQ Kit boxes.

## 2.6 Quality Control Requirements

Each batch of the naica IQ/OQ Kit is functionally tested on the QX700 ddPCR System and must perform within the established specifications. A Certificate of Compliance is available upon request from Bio-Rad Technical Support.

## Section 3: Safety Precautions and Warnings

All components referenced in this document are for *professional use in a laboratory environment only*.

**Caution:** naica Multiplex ddPCR Mix buffers are classified as hazardous according to Regulation (EC) No. 1272/2008 [CLP]. Pay particular attention to the information in the following subsections.

Contains Ovalbumins, 3(2H)-isothiazolone, 2-methyl-



### 3.1 Hazard Statements

H317: Can cause an allergic skin reaction

H334: Can cause allergy or asthma symptoms or breathing difficulties (if inhaled)

### 3.2 Precautionary Statements

P280: Wear protective gloves/protective clothing/eye protection/face protection

P302 and P352: IF ON SKIN: Wash with soap and water.

P342 and P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.

P501: Dispose of contents/container in accordance with local, regional, national, and international regulations.

### 3.3 Good Laboratory Practices

Always use Good Laboratory Practices (GLP) and adhere to the following warnings and restrictions:

- Do not eat, drink, or smoke when using this product.
- Always wear appropriate personal protection equipment for handling this product. *A lab coat, disposable gloves, and appropriate eye/face protection are required.* When necessary, wear additional personal protection equipment.
- Remove contaminated work clothing immediately. Do not remove items from the workplace until they are decontaminated.
- To prevent cross-contamination, change disposable gloves frequently and prevent cross-contamination.
- Before breaks and after work, *always wash your hands.*
- You must treat all materials of human origin as potentially infectious. Handle samples based on Standard and Universal Precautions, and follow local, regional, and national guidelines (such as *Biosafety in Microbiological and Biomedical Laboratories*). Dispose of all samples according to biohazardous and medical waste management regulations.
- Ensure there is enough ventilation to provide general biosafety.

### 3.4 Exposure Responses and Treatments

**Important:** If you feel unwell, call a doctor/physician or poison center immediately.

- In case of skin contact, wash with soap and water. If skin irritation or a rash occurs, seek medical advice/attention.
- In case of eye contact, rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. *If symptoms persist, consult an ophthalmologist.*
- If inhaled, provide fresh air.
- If swallowed, rinse the mouth.
- No special measures are necessary for self-protection of the first aider.
- In case of fire, use water, foam, or another agent suitable for ordinary combustibles.
- Clean up all spills immediately and thoroughly. Decontaminate the area for any spills that involve biohazardous materials.

For more information, refer to the naica IQ/OQ Kit Safety Data Sheets (SDS). The Safety Data Sheets are available at [bio-rad.com](http://bio-rad.com) and on request from Bio-Rad Technical Support.

### 3.5 Recycling and Disposal

Dispose of all kit components and contaminated materials appropriately and in accordance with all pertinent regulations.

Note the following:

- Waste classified as biohazardous must be disposed of in compliance with relevant laboratory, local, and/or national requirements/regulations.
- To recycle cardboard packaging, follow the requirements applicable to your laboratory or location.

## Section 4: Assay Protocol for Operational Qualification

### 4.1 Preparing the Reaction (Step 1)

The estimated reaction preparation time for the OQ run is 40 min.

Note the following:

- The OQ run for Model E and Model S consists of one run (Run 1) using three RDG16 cartridge consumables with all chambers filled with positive controls. This run qualifies the single thermal cyclers.
- The OQ run for Model HT consists of two runs (Run 1 and Run 2) using three RDG16 cartridge consumables each (for a total of six), with all chambers filled with positive controls. Each run qualifies one of two thermal cyclers.

Step	Action	Status																																	
1.	a. Thaw completely each kit reagent at room temperature (+20° C to +25° C) for up to 30 minutes. b. Vortex all tubes for 10 seconds at maximum speed. c. Spin briefly in a centrifuge to collect the contents at the bottom of each tube.	Pass Fail N/A																																	
2.	Assemble the reagents as shown in Table 4.1. The reaction mix volumes that are displayed are sufficient to process 48 chambers on either the QX700E or QX700S (Run 1) and an additional 48 chambers (for a total of 96) on the QX700 HT (Run 1 and Run 2).	Pass Fail N/A																																	
<b>Table 4.1 Reaction mix setup for the OQ run</b>																																			
<table border="1"> <thead> <tr> <th>Component</th> <th>Cap color</th> <th>Run 1, Models E and S (volume in <math>\mu\text{L}</math> for 48 rxn)</th> <th>Run 1 and Run 2, Model HT (volume in <math>\mu\text{L}</math> for 96 rxn)</th> </tr> </thead> <tbody> <tr> <td>Buffer A</td> <td>Blue</td> <td>31.7</td> <td>63.4</td> </tr> <tr> <td>Buffer B</td> <td>Red</td> <td>12.7</td> <td>25.3</td> </tr> <tr> <td>Buffer C</td> <td>Yellow</td> <td>15.8</td> <td>31.7</td> </tr> <tr> <td>Buffer D</td> <td>Brown</td> <td>15.8</td> <td>31.7</td> </tr> <tr> <td>Positive control</td> <td>Purple</td> <td>12.7</td> <td>25.3</td> </tr> <tr> <td>Nuclease-free water</td> <td>Clear</td> <td>228.1</td> <td>456.2</td> </tr> <tr> <td><b>Total volume</b></td> <td></td> <td><b>316.8</b></td> <td><b>633.6</b></td> </tr> </tbody> </table>				Component	Cap color	Run 1, Models E and S (volume in $\mu\text{L}$ for 48 rxn)	Run 1 and Run 2, Model HT (volume in $\mu\text{L}$ for 96 rxn)	Buffer A	Blue	31.7	63.4	Buffer B	Red	12.7	25.3	Buffer C	Yellow	15.8	31.7	Buffer D	Brown	15.8	31.7	Positive control	Purple	12.7	25.3	Nuclease-free water	Clear	228.1	456.2	<b>Total volume</b>		<b>316.8</b>	<b>633.6</b>
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3.	a. After assembly, vortex the reaction mix tube for 10 sec at maximum speed to mix all reagents. b. Briefly centrifuge to collect the contents at the bottom of the tube.	Pass Fail N/A																																	

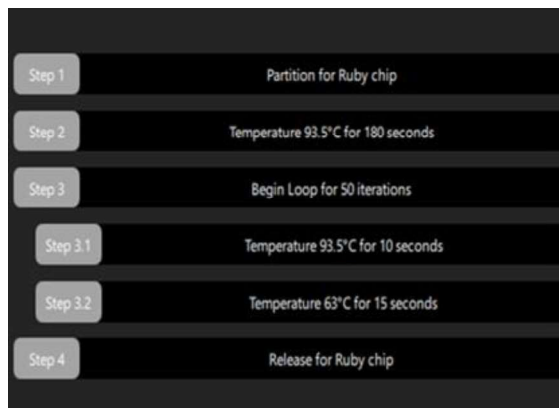
Step	Action	Status
4.	<p>(Optional) For the use of multi-channel micropipettes, transfer the reaction mix into each well of the 8-well strip tubes, as follows:</p> <ul style="list-style-type: none"> <li>• 38 <math>\mu</math>L for Model E and Model S</li> <li>• 75 <math>\mu</math>L for the Model HT</li> </ul>	Pass Fail N/A
5.	Refer to the cartridge and cartridge plate IFU (RDG16/RDG48) for instructions on the anti-static protocol and general loading procedure.	Pass Fail N/A
6.	<p>Load 5 <math>\mu</math>L of the reaction mix into each chamber of the RDG16 consumables, in total 48 or 96 chambers for the Model E/Model S and Model HT, respectively.</p> <p>Bio-Rad recommends loading the cartridge consumables immediately before starting the run on the instrument.</p>	Pass Fail N/A
Observations/Comments:		
Executor initial and date		

## 4.2 Qualifying Partition, Amplification, and Image Acquisition (Step 2)

Before you begin, download the .nioexperiment file to a USB drive from the naica IQ/OQ Kit (catalog no. 12025260) product page at [www.bio-rad.com](http://www.bio-rad.com). Note the following estimated experiment durations for partitioning, amplification, and image acquisition:

- For Model E and Model S (Run 1), 2h 45 min
- For Model HT (Run1 and Run 2), 3h, 15 min

A: Thermal cycling program parameters

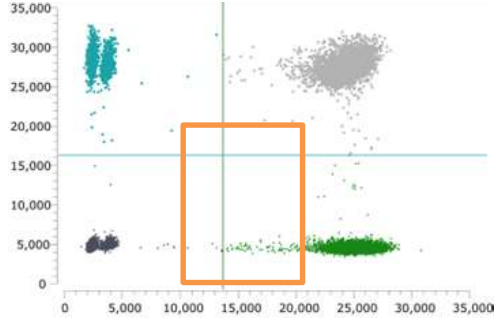
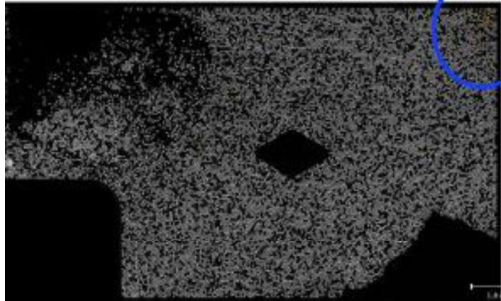


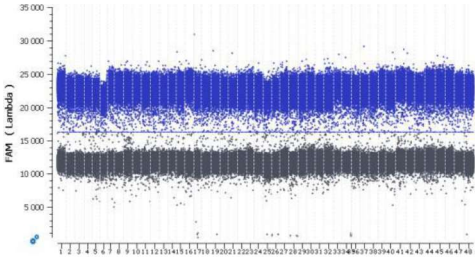
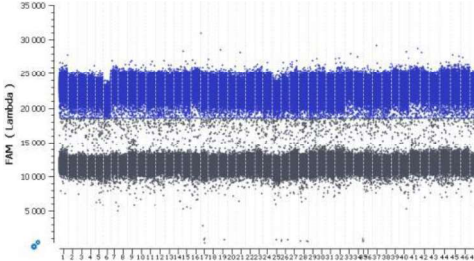
B: Reading parameters



Step	Action	Status
1.	Insert the USB drive into the USB port and then open the QX700 ddPCR System Control Software on the instrument.	Pass Fail N/A
2.	Select RUNS > Plan & Start and browse to and load the .nioexperiment file from the USB drive. <i>If this is for the second run on the Model HT, use the same file as in the first run.</i>	Pass Fail N/A
3.	Load an RDG48 cartridge holder with 3 RDG16 cartridges with chambers that have been filled with the kit components.	Pass Fail N/A
4.	Select Start Run to prompt Run 1 or Run 2 <i>Run 2 applies to Model HT only.</i>	Pass Fail N/A
5.	Select RUNS > Status to verify that the first or second cartridge holder enters the thermocycling process. <i>The second cartridge holder applies to Model HT only.</i>	Pass Fail N/A
6.	At the end of OQ run, copy the .niodata results file for Run 1 ( <i>and Run 2 for Model HT</i> ) onto a USB drive.	Pass Fail N/A
7.	To recover the plates, select RUNS > Status > Run, and then select the run.	Pass Fail N/A
8.	Select the Upload option on the right. <b>Note:</b> If necessary, you can scan the cartridge consumables again.	Pass Fail N/A
Observations/Comments:		
<b>Executor initial and date</b>		

### 4.3 Data Analysis Using QX700 ddPCR Analysis Software (Step 3)

Step	Action	Status
1.	From the naica IQ/OQ Kit (catalog no. 12025260) product page at <a href="http://www.bio-rad.com">www.bio-rad.com</a> , download the Analysis Template Excel file that corresponds to the respective naica IQ/OQ Kit lot number used for the OQ run:  AnalysisTemplate_QX700_RDG16_naicaIQOQKit_<lot-number>.xlsx	Pass Fail N/A
2.	Open QX700 ddPCR System Analysis Software and then open the Run 1 .niodata file generated by the QX700 ddPCR System Control Software.	Pass Fail N/A
3.	Click QUALITY CONTROL and check the droplet quality for all chambers in the Blue channel.	Pass Fail N/A
4.	a. Click ANALYZE DATA > Explore Crystals. b. Select the rain in the Green channel and then increase the population opacity.	Pass Fail N/A
5.	Under Channel Selection, click the Green channel and screen for droplet observations in all chambers.  <b>Important:</b> If droplet observations are present (such as air bubbles, dust particles, few analyzable droplets, and so forth, in any of the chambers, note the chamber number and refer to Section 4.4 Interpretation of Results (Step 4) for additional instructions.	Pass Fail N/A
<p><b>A: Selected Green channel rain zone</b></p> 		<p><b>B: Screen for thermal inhomogeneity in droplets</b></p> 

Step	Action	Status
6.	Select ANALYZE DATA > Plots and Populations > 1D dot plot.	Pass Fail N/A
7.	<p>Select all chambers and verify that the threshold is well-placed for all channels.</p> <p>The threshold must be centered between the mean fluorescence of the positive population and the mean fluorescence of the negative population. If necessary, manually adjust the threshold.</p> <div style="display: flex; justify-content: space-around;"> <div data-bbox="289 615 781 905"> <p><b>A: 1D dot plot with correctly placed threshold</b></p>  </div> <div data-bbox="899 615 1391 905"> <p><b>B: 1D dot plot with misplaced threshold</b></p>  </div> </div>	Pass Fail N/A
8.	<p>a. Select EXPORT and click Browse to select the output folder.</p> <p>b. Click Export to export the data (with the same name as the experiment name).</p> <p>A pop-up appears, indicating that the data has been successfully exported and shows the Excel table file and Spillover compensation file for the exported data.</p>	Pass Fail N/A
9.	Select FILE > Save to save the experiment in .nioresult format (with the same name as the experiment name).	Pass Fail N/A
10.	Double-click the exported Excel table to open the file.	Pass Fail N/A
11.	Open the Analysis Template for the lot number of the naica IQ/OQ Kit.	Pass Fail N/A

Step	Action	Status
12.	Copy the values from the Results tab in the exported Excel table to the Results_Run 1 tab in the Analysis Template.	Pass Fail N/A
13.	Copy the values from the QC_Advanced tab in the exported Excel table to the QC_Advanced_Run 1 tab in the Analysis Template.	Pass Fail N/A
14.	Select the Report_Run 1 tab in Analysis Template and check for Pass/Fail. <b>Note:</b> Disregard the Results_Run 2, QC_Advanced_Run 2, and Summary tabs unless this is a second run to test the Model HT. If that is the case, continue to step 15.	Pass Fail N/A
<b>Complete the remainder of the steps for Model HT only.</b>		
15.	a. Repeat steps 1-10 with Run 2 .niodata file generated by the QX700 ddPCR System Control Software. b. Paste the result values into the Results_Run 2 and QC_Advanced_Run 2 tabs.	Pass Fail N/A
16.	Save the Analysis Template, adding test name and date in the file name.	Pass Fail N/A
17.	Select the Report_Run 2 tab of the Analysis Template and check for pass/fail.	Pass Fail N/A
18.	a. Select the Summary tab and check for OQ run pass/fail. b. Fill in the run information.	Pass Fail N/A
Observations/Comments:		
<b>Executor initial and date</b>		

### 4.3.1 Data Analysis Acceptance Criteria

Table 4.3.1: Data analysis acceptance criteria

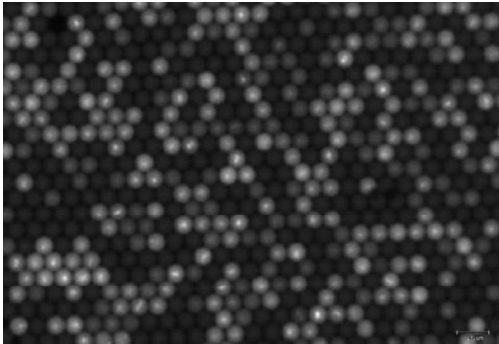
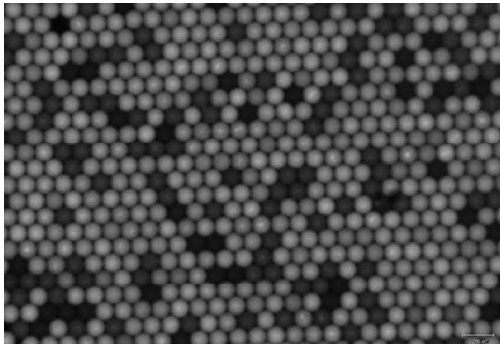
Measurement	Target Fluorophore	Imaging Channel Name	Accepted Range
Number of analyzable droplets	N/A	N/A	>10 000 per chamber
Average Concentration, IQ/OQ kit lot dependent	FAM	BLUE	Characterized value +/- 15% cp/μL
	YY	TEAL	Characterized value +/- 15% cp/μL
	ROX	GREEN*	Characterized value +/- 15% cp/μL
	ATTO-590	YELLOW*	Characterized value +/- 15% cp/μL
	Cy5	RED	Characterized value +/- 15% cp/μL
	Cy5.5	INFRA-RED	Characterized value +/- 15% cp/μL
	ATTO-550	PURPLE	Characterized value +/- 15% cp/μL
Concentration Relative Standard Deviation	FAM	BLUE	<7%
	YY	TEAL	<7%
	ROX	GREEN	<8%
	ATTO-590	YELLOW	<8%
	Cy5	RED	<8%
	Cy5.5	INFRA-RED	<7%
	ATTO-550	PURPLE	<7%
Average Separability Score	FAM	BLUE	Characterized value -15%
	HEX	TEAL	Characterized value -15%
	ROX	GREEN	Characterized value -15%
	ATTO-590	YELLOW	Characterized value -15%
	Cy5	RED	Characterized value -15%
	Cy5.5	INFRA-RED	Characterized value -15%
	ATTO-550	PURPLE	Characterized value -15%
Separability Score Relative Standard Deviation	FAM	BLUE	<15%
	HEX	TEAL	<13%
	ROX	GREEN	<10%
	ATTO-590	YELLOW	<11%
	Cy5	RED	<15%
	Cy5.5	INFRA-RED	<11%
	ATTO-550	PURPLE	<12%

\*The average concentration of the target in the Yellow and Green channels, from at least 43 chambers, is not an obligatory pass or fail criterion. Instead, it is a criterion for reagent variability monitoring and the Analysis Template displays "Warning" rather than "Fail" for the average concentration.

#### 4.4 Interpreting the Results (Step 4)

Step	Action	Status
1.	Confirm that the correct version of the Analysis Template Excel file corresponding to the respective naica IQ/OQ Kit lot number is available and will be used for accurate interpretation of results.	Pass Fail N/A
2.	The OQ run data are validated if: <ul style="list-style-type: none"> <li>The average total number of droplets in each chamber is within the acceptance criteria</li> <li>The target concentrations that were averaged from at least 43 chambers are within the acceptance range</li> <li>The relative standard deviations of the target concentrations are within the acceptance criteria</li> <li>The separability scores that were averaged from at least 43 chambers are within the acceptance criteria</li> <li>The relative standard deviations of separability scores are within the acceptance criteria</li> </ul>	Pass Fail N/A
3.	<b>For Model E and Model S only:</b> If all acceptance criteria for Run 1 display PASS, select Conform from the OQ Decision dropdown menu in the Report_Run 1 tab.	Pass Fail N/A
4.	<b>For Model HT only:</b> If all acceptance criteria for the Run 1 and Run 2 display PASS, select Conform from the OQ Decision dropdown menu in the Report_Run 2 tab. <b>Note:</b> For Model HT, the Conform status applies only if both Run 1 and Run 2 pass. In this case, Conform appears on the global OQ Decision in the Summary tab of the Analysis Template.	Pass Fail N/A

Step	Action	Status
5.	If at least one of the acceptance criteria displays FAIL or droplet observations exist in any of the chambers, complete the following substeps:	
	a. In the Analysis Template, identify the individual cartridge chambers that are creating interpretation bias of the OQ result. Cross-check if chambers creating bias match noted chambers containing droplet observations.	Pass Fail N/A
	b. To check for and confirm the presence of droplet observations, open the .nioresults file and click QUALITY CONTROL to display the droplet images. For information and instructions, see Section 4.4.1, Thermal Inhomogeneity.	Pass Fail N/A
	c. Refer to the troubleshooting section in the instrument guide for troubleshooting steps on droplet observations.	Pass Fail N/A
	d. After troubleshooting, if no thermal inhomogeneity is observed, the user can exclude from analysis up to five (5) chambers per OQ run. Clear each chamber to be excluded (select 0 in the Exclude chambers column) and note the justification of the exclusion in the dedicated space of the Analysis Template. <i>For better traceability, do not remove the affected chamber from the .nioresult file.</i>  <b>Note:</b> Chambers in position A and H cannot be excluded by pairs within the same cartridge (for example, A1-A2 or H1-H2) from the Analysis Template for a qualification. In case of droplet observations in chamber pairs A1-A2 or H1-H2, run the OQ run again. For more information, see Section 4.4.1, Thermal Inhomogeneity.	Pass Fail N/A
	e. Reassess the Report tab of the Analysis Template and check for pass/fail.	Pass Fail N/A
	f. Do one of the following: <ul style="list-style-type: none"> <li>• If PASS appears for all acceptance criteria, select Conform from the OQ Decision dropdown menu and finalize the OQ documentation.</li> <li>• If FAIL appears for at least one of the acceptance criteria after excluding the chamber(s) without thermal homogeneity, select Not Conform from the OQ Decision dropdown menu and contact Bio-Rad Technical Support</li> </ul> <p>The average separability scores of the targets detected in the Purple and Infra-Red channels are sensitive to the presence of droplet observations, particularly the presence of fluorescence aggregates (see figures A and B in Step 6e). When average separability scores for the targets are close to the acceptance criteria limit, the Analysis Template displays <i>Check for the presence of Droplet Crystal observations.</i></p>	Pass Fail N/A

Step	Action	Status
6.	To check for the presence of Droplet Crystal observations:	
	a. Open the .nioreult file and select QUALITY CONTROL.	Pass Fail N/A
	b. Select the channel of interest; for example, Purple and Infra-Red channels.	Pass Fail N/A
	c. For a closer inspection of the droplet quality, clear the Show All checkbox.	Pass Fail N/A
	d. If droplet observations are not detected, no additional action is required.	Pass Fail N/A
	<p>e. If droplet observations are detected, add a note in the dedicated space of the Analysis Template for traceability purposes.</p> <p>A: Example of fluorescent aggregates</p>  <p>B: Example of subtle fluorescent aggregates</p> 	Pass Fail N/A
Observations/Comments:		
Executor initial and date		

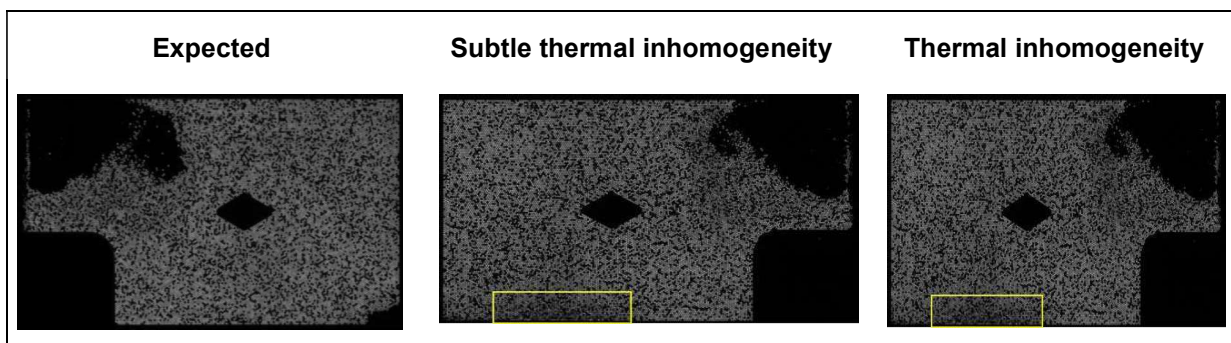
### 4.4.1 Thermal Inhomogeneity

The naica IQ/OQ Kit is designed to detect thermal inhomogeneity of the thermal cycler(s) as part of this OQ protocol. Thermal inhomogeneity is the absence of amplification or delayed amplification in droplets due to insufficient heating, which can be caused by a deviation from the optimal calibration of the QX700 ddPCR System thermal block peltiers. Absent or delayed amplification causes a lower fluorescence in the droplet, which results in a dark patch or zone in the droplet image and rain in the 1D dot plot.

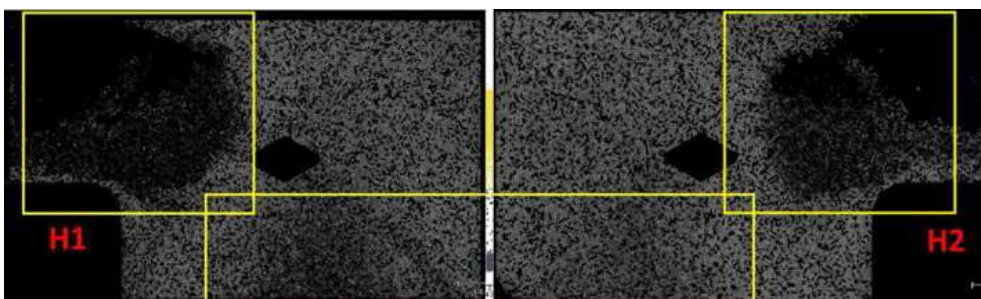
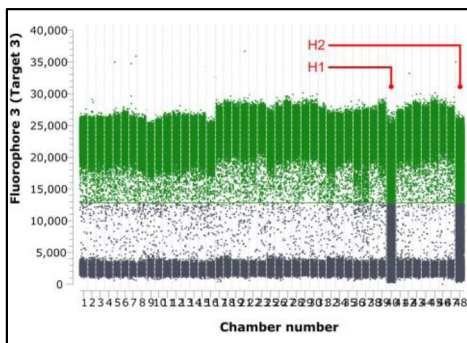
Due to the configuration of the QX700 ddPCR System, thermal inhomogeneity frequently occurs in chambers A and H of the RDG16 cartridge. It is frequently located on the top and bottom of the droplet, and occasionally on the right edges (cartridge column 1) and left edges (cartridge column 2). Therefore, you should examine all chambers to verify thermal homogeneity or inhomogeneity.

#### To determine thermal inhomogeneity

1. Inspect the droplet images in the affected chambers. Use the following figures as examples for comparison.



2. In case of suspected thermal inhomogeneity in the droplet images, inspect 1D dot plots of the Green channel.



3. If thermal inhomogeneity is detected in either run, select Not Conform from the OQ Decision dropdown menu and contact Bio-Rad Technical Support.

## Section 5: Protocol Exception and Actions Taken

If an exception or deviation has been made to this protocol, or if a part requires replacement, the executor must comment on the reason for the exception and list the action that was taken to correct the situation. The executor should provide a description, including the section in which the problem occurred and/or any parts replaced.

**Table 5: Protocol exceptions and actions taken**

Exceptions		
1.		
2.		
3.		
Actions Taken		
1.		
2.		
3.		
Executor Name	Signature	Date

## Section 6: Attachment Log Summary

A detailed summary of all the attachments (data, instrument printouts, logs, certification training, and so forth) shall be generated and listed below. All attachments shall be paginated and the pagination shall be indicated in this summary.

**Table 6: Attachment log summary**

Attachment	Page __ of __
Executor initial and date	

## Revision History

Release Date (Month, Year)	SAP Version	Description
October, 2025	C	Two corrections in procedural info: *changed "with" thermal homogeneity to "without" in Step 5f of Interpreting the Results *added 38 $\mu$ L to Step 4 of Preparing the Reaction
September, 2025	B	Incorporate SME edits, remove signature blocks
August, 2025	A	New Bio-Rad document containing information and instructions for installation and operational qualification of the QX700 ddPCR System.

## 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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