



Geode
part of the naica® system



User Manual
Geode H15000
Software version 3.3.0
Sapphire Chip & Ruby Chip

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1 Introduction to Crystal Digital PCR®

1.1 Purpose of the document

This document provides general information for the use of the Geode for Crystal Digital PCR®. The workflow and the different hardware components are described in detail. It is essential to read the User Manual carefully and pay attention to the safety information provided. The instructions and safety information in the User Manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

All documents referenced in this User Manual can be accessed here:
<https://www.stillatechnologies.com/technical-resources/naica-system-prism3/>

1.2 Overview of the Crystal Digital PCR® Workflow

Crystal Digital PCR® is Stilla Technologies' next-generation technology for the absolute quantification of nucleic acids.

Using cutting-edge microfluidic innovations, this technology integrates the digital PCR process in a single consumable (Figure 1). The sample is first flowed through a network of microchannels and partitioned into a large 2D array of 12,900 to 30,000 individual droplets (Sapphire Chip)¹ and 10,000 to 17,000 droplets (Ruby Chip)², also called a Droplet Crystal. PCR is then performed within the chips and the Droplet Crystal is imaged to reveal the droplets that contain amplified targets. The last step consists of counting the number of these positive droplets to precisely extract the absolute quantity of nucleic acids.

With Crystal Digital PCR®, the combination of powerful image analysis and intuitive visual inspection offers an unmatched level of confidence in the digital PCR measurement, yielding data you can trust.

¹ 12,900 to 25,800 for naica® multiplex PCR MIX and naica® PCR MIX
15,000 to 30,000 for PerfeCTa® qPCR ToughMix®, PerfeCTa® qPCR ToughMix® UNG, PerfeCTa® Multiplex qPCR ToughMix® and qScript™ XLT One-Step RT-qPCR ToughMix®

² naica® multiplex PCR MIX and naica® PCR MIX




SAMPLE LOADING	GENERATION OF DROPLET CRYSTAL AND PCR	READING AND ANALYSIS
		
<p>Prepare the sample for the reaction mix. Stilla Technologies recommends the use of the naica® PCR MIX reagents, specifically developed for Crystal Digital PCR®. Load the reaction mix into the wells of the selected chip, and seal with the provided caps.</p>	<p>Place the prepared chips into the Geode. Launch the combined partitioning and amplification program; Droplets are generated by partitioning of each sample and PCR amplification is performed immediately after Droplet Crystal generation.</p>	<p>After PCR, transfer the chips to the Prism3 instrument. Set up the read-out using Crystal Reader software for data acquisition of the Droplet Crystal in up to 3 fluorescent channels (Blue, Green, and Red). Image analysis and data extraction are performed using the Crystal Miner software.</p>

Figure 1: Overview of Crystal Digital PCR® workflow with naica® 3-color system




SAMPLE LOADING	GENERATION OF DROPLET CRYSTAL AND PCR	READING AND ANALYSIS
		
<p>Prepare the sample for the reaction mix. Stilla Technologies recommends the use of the naica® PCR MIX reagents, specifically developed for Crystal Digital PCR®. Load the reaction mix into the wells of the selected chip, and seal with the provided caps.</p>	<p>Place the prepared chips into the Geode. Launch the combined partitioning and amplification program; Droplets are generated by partitioning of each sample and PCR amplification is performed immediately after Droplet Crystal generation.</p>	<p>After PCR, transfer the chips to the Prism6 instrument. Set up the read-out using Crystal Reader software for data acquisition of the Droplet Crystal in up to 6 fluorescent channels (Blue, Teal, Green, Yellow, Red, and Infra-Red). Image analysis and data extraction are performed using the Crystal Miner software.</p>

Figure 2 - Overview of Crystal Digital PCR® workflow with naica® 6-color system.

1.3 Intended use of the naica® system

The naica® system for Crystal Digital PCR^R is composed of two instruments: the Geode, which performs Droplet Crystal generation and amplification, and a reading instrument, the Prism3 or Prism6, which enables imaging of the droplet crystals.

The Crystal Reader software functions as the user interface to set up the experiment for the reading instrument (Prism3 or Prism6). Crystal Reader :

- Allows for the definition the analytical context of the experiments. Experiments can be set up on-demand or dedicated experimental templates can be created for recurring experimental setups.
- Controls the reading instrument (Prism3 or Prism6) for the acquisition of the fluorescence images of Sapphire Chip or Ruby Chip.
- Applies pre-analysis treatments to the acquired images and provides a first quality control in preparation for the detailed downstream experiment analysis performed by the Crystal Miner software.

The Crystal Miner software is used to extract data from the images acquired using the reading instrument (Prism3 or Prism6) and Crystal Reader software and to calculate the absolute concentrations of the targeted nucleic acids.

Crystal Reader and Crystal Miner software are pre-installed on the naica® PC delivered with the naica® system.

The naica® system performs Crystal Digital PCR^R within microfluidic chip consumables (Sapphire Chip & Ruby Chip).

The naica® PCR MIX reagents are recommended for use to achieve optimal Crystal Digital PCR^R performance on the naica® system.

For detailed instructions for Prism3, Prism6, Crystal Reader software and Crystal Miner software, please refer to the respective User Manuals. For detailed instructions for Sapphire Chip, Ruby Chip, naica® PCR MIX, and naica® multiplex PCR MIX, please refer to the respective Instruction for Use (IFU) documents.

The naica® system is a laboratory instrument to be used by qualified personnel in a controlled environment. Before using the naica® system, the user must be trained by a Stilla Technologies representative.

In general, Crystal Digital PCR^R can be performed on the naica® system with any type of nucleic acid sample. However, individual sample-type compatibility for digital PCR applications may require a dedicated assay validation by the end-user. Please note also that both sample purity and the extraction method used can influence sample compatibility for digital PCR applications.

The Geode is intended for use by professional users trained in molecular biological techniques and in the operation of the Geode.

The Geode is a part of the naica® system. The naica® system is intended for Research Use Only. Not for use in diagnostic procedures.

1.4 Citing the naica® system in scientific publications, presentations, seminars, etc.

To cite the use of the naica® system use:

Crystal Digital PCR® (Stilla Technologies, France)
naica® system (Stilla Technologies, France)
naica® system component names:

- Geode
- Prism3
- Prism6
- Sapphire Chip
- Ruby Chip
- Crystal Reader software
- Crystal Miner software
- naica® PCR MIX reagents
 - naica® PCR MIX
 - naica® multiplex PCR MIX.

2 Materials and Equipment

2.1 Geode packaging

- Geode packaging:
 - The main instrument (H15000) with the magnetic frame for Sapphire Chip
 - Certificate of Conformity
 - naica® system accessory box:
 - Magnetic Ruby Chip adapters for the Geode lid
 - Magnetic Ruby Chip frame for the Geode thermal plate
- Peripheral box:
 - Power cable for Geode
 - Compressed air system (ISPTLAPG3) including power cable
 - Precision Wipes (Kimtech™ Science, 7552, 1 ply, 213x114 mm)
 - Antistatic wetted wipes (ACL Staticide®, Reference: SW12)







Note

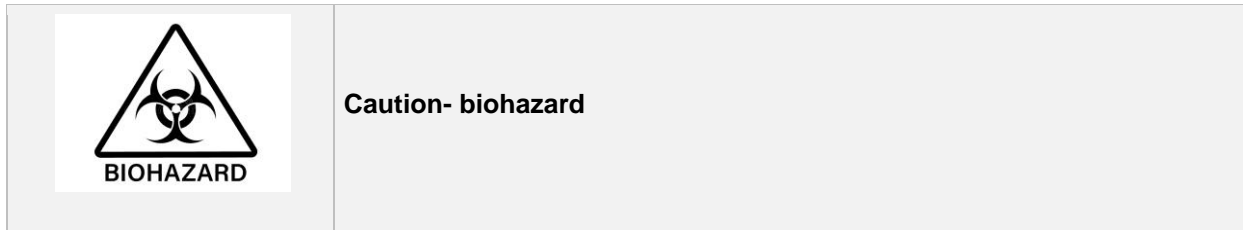
- *Replacement Precision Wipes must be purchased using the reference- Precision Wipes (Kimtech™ Science, 7552, 1 ply, 213x114 mm) from global standard laboratory suppliers*
- *Replacement Antistatic wetted wipes (ACL Staticide®, Reference: SW12) must be ordered directly from the supplier Digi-Key using the reference ST1059-ND. [SW12 ACL Staticide Inc | Static Control, ESD, Clean Room Products | DigiKey](#)*

2.2 Warning Labels

2.2.1 Safety information

The following warning labels are located on the Geode:

SYMBOL	MEANING
	<p>Caution of dangerous voltage Please ensure that the voltage indicated on the back of the device exactly matches the local electrical supply.</p>
	<p>Caution of dangerous explosive material Explosive or reactive material must not be heated or placed under pressure in the Geode.</p>
	<p>Caution- liquids Ensure that no liquids can enter the device. Sample must be loaded outside of the Geode.</p> <p>Caution- environment The ventilation of the device must not be covered.</p> <p>Caution- use If the device is used in a manner not described in this manual, safety may be compromised.</p>
	<p>Caution- hot surface The thermal plate and chips quickly reach temperatures above 50°C thus, there are risks of burning or scalding if the equipment is not operated properly. Keep the lid closed until the temperature reaches 30°C or less. Only use materials (chips and caps) provided by Stilla Technologies, which are heat resistant at temperatures up to 95°C.</p>
	<p>Caution- the risk of pinching The lid of the Geode should be closed with caution before starting a run. Do not try to open the Geode lid during a run. Do not manipulate the Geode lid handle during a run. Always check that the internal pressure of the chamber is below 20 mbar before opening the lid.</p>
	<p>Protective conductor terminal</p>



2.2.2 General safety instructions: Geode and compressed air system










General safety instructions for the Geode and the compressed air system (ISPTLAPG3):

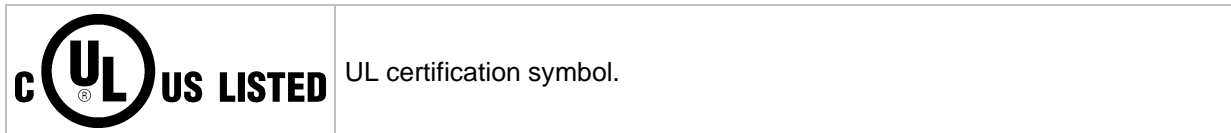
- Please ensure that the Geode is operated as instructed in the provided User Manuals. User Manuals are subject to changes. The latest version can be accessed from the Technical Resources webpage:
<https://www.stillatechnologies.com/technical-resources/naica-system-prism3/>.
- Stilla Technologies cannot be held responsible for any damages or injuries arising from improper operation.
- Do not use the instruments if any parts are broken, chipped, rusty, or if the power cables are damaged in any way.
- Do not open the housing of the Geode and the compressed air system (ISPTLAPG3).
- Opening the Geode or the compressed air system (ISPTLAPG3) housing may breach any warranty for the instruments.
- Only operate the Geode and the compressed air system (ISPTLAPG3) with the provided and specified detachable main supply cords. Do not replace the components with non-specified cords.
- Do not attempt any repairs or alterations except as expressly instructed in the User Manual or by a Technical Support Representative.
- Always disconnect the instruments from their power source before cleaning or moving the Geode instrument and the compressed air system (ISPTLAPG3).
- For the Geode and the compressed air system (ISPTLAPG3), the mains supply source must meet the national regulatory requirements.
- The power cord of the Geode and the compressed air system (ISPTLAPG3) must be connected to a wall outlet with a grounded conductor.
- For the Geode and the compressed air system (ISPTLAPG3), the mains voltage must correspond to the range given in the product specification.
- Keep liquids away from the Geode and the compressed air system (ISPTLAPG3); avoid percolation of liquids inside the instrument.
- For optimal use of the Geode, the room temperature should be between 15°C and 25°C.
- Samples can be infectious or cause other damage to health. Safety regulations issued for the handling of sample material in the laboratory must be followed by wearing the proper Personal Protective Equipment (e.g., gloves and protective clothing). For detailed instructions, please refer to the Decontamination section of this manual.
- Mains supply voltage 110V (+/- 10%) or 240V (+/- 10%).

- Stilla Technologies does not recommend moving the Geode after installation as moving affects the calibration of the Geode. If for any reason the Geode has to be moved, please ensure to follow the steps listed below:
 - For lifting the Geode- get as close to the load as possible. Try to keep your elbows and arms close to the body. Keep the back straight while lifting by tightening the core muscles, bending at the knees, keeping the load close, centered in front, and look up and ahead. Get a good handhold and do not twist while lifting. Do not jerk; use a smooth motion while lifting. If the load is too heavy to allow this, seek help to lift.
 - Carrying the Geode- do not twist or turn the body; instead, move the feet to turn. The hips, shoulders, toes, and knees should face the same direction. Keep the load as close to the body as possible with the elbows close to the body. If fatigued, set the load down and rest for a few minutes. Do not excessively strain while trying to lift the Geode, the proper posture should be maintained for setting down and lifting technique.
 - Setting down the Geode- set the load down in the same way it was picked up, but in the reverse order. Bend at the knees, not the hips. Keep the head up, the core muscles tight, and do not twist the body. Keep the load as close to the body as possible. Wait until the load is secure to release your handhold.
- The Geode must be placed on a stable, solid, and vibration-free surface.
- The Geode should not be exposed to direct sunlight.
- There should be enough space to make sure that the fans at the front, the back, or the sides are not covered and that the main switch is accessible. There should be a distance of at least 25 cm between the instrument and any wall or neighboring objects. Multiple Geodes should not be placed directly next to each other either back-to-back or back-to-front.
- No objects should be placed on top of the Geode.
- If a part of the Geode chamber breaks while a 1 bar overpressure is building up, a sudden noise or pop can be generated for a fraction of a second without causing any harm to the user.
- If a part of the Geode chamber loosens or breaks, then the Geode is not leakproof. Pressure cannot build up so that there is no chance of any sudden noises or of any parts being projected to cause harm.

2.3 Geode labeling

2.3.1 Labeling symbols

SYMBOL	MEANING
	Manufacturer.
	Product reference (part number).
	Product serial number.
	Read the User Manual before using the product.
	Caution: documentation must be consulted in all cases where this symbol is marked. Using the product without applying the instructions detailed in the User Manual may result in personal injury or damage to the equipment and facilities.
	Alternating current.
	Restriction of Hazardous Substances (Directive 2002/95/EC on the restriction of the use of certain hazardous substances in electrical and electronic equipment).
	The product should be disposed of in an appropriate recovery and recycling structure.
	CE marking (manufacturer's declaration that the product meets the requirements of the applicable EC directives).



2.4 Geode declaration of conformity

To view the declaration of conformity certificate, please visit the Technical Resources webpage.

2.5 Geode specifications

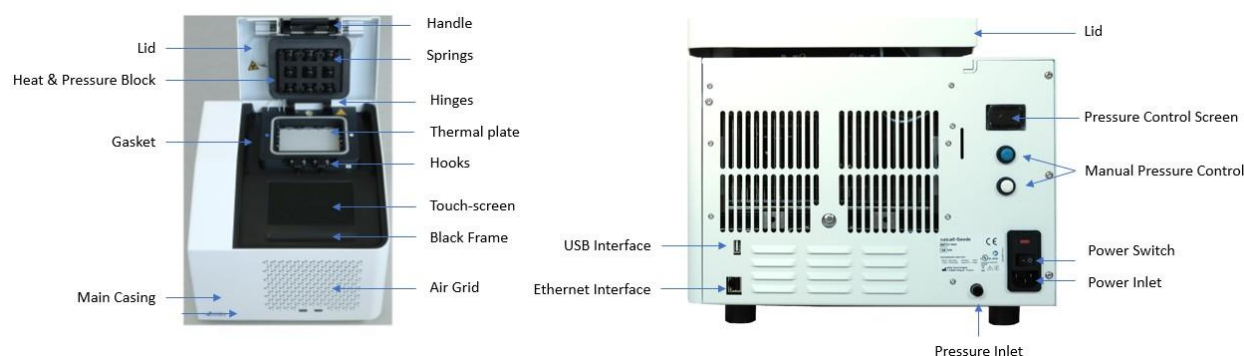


Figure 3: Front (A) and back (B) view of the Geode. USB ports and intended for data import or export.

TECHNICAL SPECIFICATIONS	
Capacity	Up to 12 samples (Sapphire Chip) or 48 samples (Ruby Chip); 3 chips/run
Thermal block operational temperature range*	10 °C to 95 °C
Control accuracy thermoblock	± 0.1 °C
Block uniformity (at 72 °C)	± 1.0 °C
Adjustable ramping	from 0.1°C/s to 2°C/s
Pressure in-out	0 – 1.3 bar
Pressurizing gas	Air
Screen	Touch-sensitive TFT-Display (VGA, Graphics, 65535 colors)
Functional interfaces	3 x USB
Dimensions (W x D x H)	35 x 37 x 29 cm
Weight	16 kg ± 0.5 kg
Power supply	100-240V~ // 50/60Hz // 750W
Fuse	T10AH/250V~
Noise level	< 37 dB (A)
Disconnection device	The plug socket serves as a disconnecting device; the plug socket must be easily accessible.
Maintenance	All parts are required to be examined or supplied only by the manufacturer or its agent.

ENVIRONMENTAL IMPACT	
Pollution degree	2
OPERATIONAL CONDITIONS	
Environmental conditions	Indoor use only
Temperature	+15 °C to +25 °C
Relative humidity	Max. 70%
STORAGE CONDITIONS	
Temperature	+5°C to +40°C
Relative humidity	10% to 95% non-condensing
Barometric pressure	700 hPa to 1060 hPa
Waiting time after plugging in before operating	8h
SHIPPING CONDITIONS	
Temperature	-40°C to +60°C
Relative humidity	10% to 95% non-condensing
Barometric pressure	700 hPa to 1060 hPa
Waiting time until operation	24h

*The thermal block in the Geode can be set to operate at temperatures ranging from 10 to 95°C. However, the thermal plate temperature should never be set below the indicated operational temperature (room temperature). Use outside of the indicated operational temperatures exposes the Peltier elements that regulate the temperature of the thermal plate to significant damage and could reduce the operating life of the instrument

2.6 Geode configuration and chip compatibility information

Two microfluidic chip consumables are compatible with the naica® system: Sapphire Chip and Ruby Chip. Please refer to the **Error! Reference source not found.** below for Geode instrument/software/chip consumable compatibility information.

Table 1: Compatibility matrix between the chip consumables and the different versions of the Geode instrument and the different software versions

GEODE VERSION	SOFTWARE VERSION	SAPPHIRE CHIP COMPATIBILITY	RUBY CHIP COMPATIBILITY
H15000	3.3.0	Yes	Yes
	3.2.0	Yes	Yes Hardware upgrade might be required
	3.1.0 & 3.1.1	Yes	Software upgrade required. Hardware upgrade might be required
H14000	2.7.0	Yes	Software and hardware upgrade required
	2.6.0	Yes	Software and hardware upgrade required
H13000	2.7.0	Yes	Software and hardware upgrade required
	2.6.0	Yes	Software and hardware upgrade required

2.7 Installation of the Geode

2.7.1 Installation environment requirements

Installation requirements for the Geode include:

- clean laboratory environment
- stable, solid, and vibration-free surface. Surface capable to support between 20 to 40 kg
- a minimum distance of 25 cm to neighboring objects around the Geode.
- room temperature between 15°C and 25°C

2.7.2 Installation certification required to operate naica® system

The initial naica® system installation must always be executed by a Stilla Technologies representative.

The naica® system installation and operational qualification according to Stilla Technologies specifications will be documented by an installation report. The report is required to release the naica® system for customer operation.

2.8 Pre-requisite information prior to Crystal Digital PCR® experiments

To detect and quantify the amount of target in the sample with the naica® system, a fluorescent signal is measured. The Crystal Digital PCR® technology developed by Stilla Technologies allows the use of fluorescently labeled hydrolysis probes (TaqMan®) as well as of the non-specific fluorescent intercalating dye EvaGreen®.

Please note that SYBR® Green is not compatible with the naica® system.

A fluorophore is a fluorescent chemical compound that absorbs light energy of a specific wavelength and emits light at a longer wavelength. Fluorophores are routinely used in many applications and are notably characterized by their excitation/emission spectra.

2.8.1 Crystal Digital PCR® recommended detection system for 3-color naica® system

In the 3-color naica® system configuration / Prism3 (H22000), detection in three channels is available.

To be detected by the Prism3 instrument, the respective fluorophore excitation and spectra must overlap with that of the LED of a given detection channel, and its emission spectrum must fall within the bandpass of the tri-band filter used for acquisition (Table 2).

Table 2: The Prism3 optical specifications and examples of compatible fluorophores.

	BLUE CHANNEL	GREEN CHANNEL	RED CHANNEL
Excitation range	415-480 nm	530-550 nm	630-640 nm
Emission range	495-515 nm	560-610 nm	655-720 nm
Compatible fluorophores (reporter)	FAM, Fluorescein	Cy®3, HEX	Alexa Fluor® 647, Cy®5

Note: One fluorophore might be excited by several LEDs and emit light in different acquisition channels. This phenomenon can readily be corrected by applying compensation post-image acquisition (refer to the specific Crystal Miner software User Manual for details).

2.8.2 Crystal Digital PCR® recommended detection system for 6-color naica® system

In the 6-color naica® system configuration / Prism6 (H24000.6), detection in Blue, Teal, Green, Yellow, Red and Infra-red channels is available.

To be detected by the Prism6 instrument, the respective fluorophore excitation and emission spectra must overlap with the specified bandpass of the instrument for image acquisition (**Table 1**).

Table 3 – The Prism6 optical specifications and examples of compatible fluorophores.

	BLUE CHANNEL (nm)	TEAL CHANNEL (nm)	GREEN CHANNEL (nm)	YELLOW CHANNEL (nm)	RED CHANNEL (nm)	INFRA-RED CHANNEL (nm)
Excitation range	445-490	504-526	540-560	562-588	623-643	675-698
Emission range	503-537	527-551	566-597	598-642	650-684	704-755
Compatible fluorophores (reporter)	FAM, Fluorescein	Yakima Yellow®	Atto 550	ROX	Cy®5	Cy®5.5

Note: One fluorophore might be excited by several LEDs and emit light in different acquisition channels. This phenomenon can readily be corrected by applying compensation post-image acquisition (refer to the specific Crystal Miner software User Manual for details).

2.8.3 The reference dye

In Crystal Digital PCR®, reference dyes are used to increase the basal fluorescence of droplets in the blue channel and thus enable their detection by the Prism3 and the Crystal Reader software.

Fluorescein is the reference dye for TaqMan® based assays. The naica® multiplex PCR MIX already contains the reference dye and does not therefore require the addition of extra fluorescein.

Fluorescein should however always be added to the reaction mix when using PCR mixes other than the ready-to-use naica® multiplex PCR MIX for fluorescent probe-based assays (e.g. Taqman® probes). This addition will thus ensure successful Crystal Digital PCR® by allowing for droplet recognition in the blue channel.

For EvaGreen® based assays, the basal fluorescence from the EvaGreen® dye is usually sufficient to allow droplet detection in the blue channel. However, the basal fluorescence signal is not always sufficient for optimal droplet detection and an alternative reference dye must be used. Adding Alexa Fluor® 647 as a reference dye allows for droplet recognition in the red channel without disturbing the EvaGreen® signal detected in the blue channel. Stilla Technologies therefore recommends the addition of Alexa Fluor® 647 as a reference dye to all EvaGreen® experiments.

Please check that the droplets have been correctly detected when using a new batch of the reference dye.

2.8.4 Crystal Digital PCR® recommended PCR mastermixes

Note – materials required but NOT supplied with naica® system, order separately based on specific application.

Depending on the application, Stilla Technologies recommends the use of:

- naica® multiplex PCR MIX for fluorescently labeled probe-based digital PCR assays (TaqMan®)
- naica® PCR MIX for EvaGreen®

These dye-based reagents will give optimal results for Crystal Digital PCR® on the naica® system. These reagents are available at 5X and 10X concentrations and Stilla Technologies provides fast cycle or conventional PCR cycling protocols for initial protocol development. More information is available at: <https://www.stillatechnologies.com/naica-system-dpcr-mixes/>.

For RT-PCR applications Stilla Technologies recommends the use of qScript™ XLT One-Step RT-qPCR ToughMix® (QuantaBio).

When using cDNA, Stilla Technologies recommends using the qScript® Flex cDNA Synthesis kit (QuantaBio).

Any other enzymatic mix needs to be evaluated by the user before performing experiments, as Stilla Technologies cannot guarantee the compatibility of any other enzymatic mix with the naica® system.

2.8.5 PCR primers

Note – materials required but NOT supplied with naica® system, order separately based on specific application.

Note – materials required but NOT supplied with naica® system, order separately based on specific application.

In genomic applications, a primer is a short single-stranded DNA fragment used in polymerase chain reaction (PCR) technologies. A pair of primers hybridizes with the sample DNA and defines the region that will be amplified by initiating the DNA synthesis.

Please take notice of the available Technical Note [Guidelines for 3-color multiplex assay design for optimized performance with Crystal Digital PCR® | Stilla Technologies](#)

2.8.6 TaqMan® probes

Note – materials required but NOT supplied with naica® system, order separately based on specific application.

TaqMan® probes consist of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end. Several different fluorophores and quenchers are available. The quencher molecule quenches the fluorescence emitted by the fluorophore when excited by the light source. As long as the fluorophore and the quencher are in proximity, quenching inhibits any fluorescence signals.

2.8.7 Non-specific fluorescent intercalating dye: EvaGreen®

Note – materials required but NOT supplied with naica® system, order separately based on specific application.

Intercalating dyes can also be used with Crystal Digital PCR®, for example EvaGreen®. EvaGreen® is a non-mutagenic and non-cytotoxic DNA-binding dye that is compatible with Crystal Digital PCR®. Non-fluorescent when free in solution, it becomes strongly fluorescent upon binding to double-stranded DNA (dsDNA) in a sequence-independent manner. The signal generated can readily be detected using the same filters as those used for FAM or fluorescein (blue channel).

Coupled with Crystal Digital PCR®, EvaGreen® thus enables absolute quantification of a target by using a simple primer pair.

3 Setting up a Crystal Digital PCR® experiment

3.1 Definition of a Crystal Digital PCR® experiment

An experiment is defined as being a set of samples processed during an individual Crystal Digital PCR® workflow on the naica® system:

- The samples of an individual experiment were all processed on the same respective chip type (Sapphire Chip or Ruby Chip). Chip types cannot be mixed in a single experiment for partitioning and amplification in the Geode.
- The samples of the individual experiment were prepared using the same PCR mastermix and the same PCR program for partitioning and amplification in the Geode.

3.2 Preventing DNA contamination

Due to the extraordinary sensitivity of most Taq polymerases, exogenous DNA amplification can occur and bias an entire experiment (Kwok and Higuchi, 1989). Since many sources of contamination exist such as cross-contamination and previous PCR amplification, it is essential to adhere to a strict set of protocols combined with certain precautions.

To limit DNA contamination, the following conventional recommendations for PCR should be followed (not exhaustive):

- Use separate areas and dedicated equipment/supplies for sample preparation, PCR setup, PCR amplification, and analysis of PCR products. Wear a clean lab coat and gloves during each step of the experiment.
- Clean laboratory benches and equipment periodically with water/ethanol, or with 'Dnase/Rnase away' depending on the application (avoid aerosols of cleaning reagents to prevent contamination).

Note: Bleach is not recommended; if its use is unavoidable, be sure to abundantly rinse with water after the bleach treatment.

- Keep tubes capped as much as possible.
- Centrifuge tubes after vortexing to limit aerosols.
- Monitor potential contaminants by including a no template control in each run.
- Consider aliquoting reagents if possible, to prevent contamination and, at the same time, to reduce repetitive freezing/thaw cycles that might affect their efficiency.

3.3 Protocol for setting up Crystal Digital PCR®

3.3.1 Sample purification and pre-treatment

A range of extraction kits and methods can be used to purify nucleic acids from a variety of sample types. However, individual sample-type compatibility for digital PCR applications may require a dedicated assay validation by the end-user.

DNA samples with ≥ 10 kb average length (e.g., genomic DNA) should be fragmented by restriction digestion before partitioning to ensure even distribution of the DNA template during partitioning. Restriction digestion is not required for highly fragmented DNA (e.g., FFPE DNA or circulating DNA). Care must be taken to use restriction enzymes that do not cut within the amplified sequence.

4 Reaction mix preparation

For Crystal Digital PCR®, Stilla Technologies recommends using the naica® PCR MIX reagents.

For other compatible PCR Master mix compositions, refer to the IFU for additional validated Crystal Digital PCR® qPCR reagents for the 3-color and 6-color naica® system.

Always thaw each reagent, vortex, and spin briefly in a microcentrifuge to collect the material in the bottom of the tube.

4.1 Fluorescently labeled probes

When using fluorescently labeled TaqMan® probes, Stilla Technologies recommends using the naica® multiplex PCR MIX.

For the reaction mix composition using the naica® multiplex PCR MIX, view the detailed reaction preparation information in the corresponding IFU.

Please refer to the next section for guidelines on primer and probe concentrations.

Vortex and centrifuge the reaction mix after adding the reagents to avoid air bubbles that may compromise the Crystal Digital PCR® reaction.

4.2 Non-specific fluorescent intercalating dye: EvaGreen®

When using EvaGreen®, Stilla Technologies recommends using the naica® PCR MIX.

For the reaction mix composition using the naica® PCR MIX, view the detailed reaction preparation information in the corresponding IFU.

5 Optimization of Crystal Digital PCR®

5.1 Fluorescently labeled probes

The optimal primer and probe concentrations as well as the optimal hybridization/elongation temperature during the cycling should notably be tested. Optimal results involve good separability between positive and negative droplets, i.e. no or few “rain” droplets (positive droplets with variable intensity localized between the negative and positive cluster) that could be due for example to non-specific hybridization of the primers/probe or competition phenomena. Only if you have good separability will you have high quality concentration measurements.

In addition, the efficiency of singleplex reactions should always be evaluated first before proceeding to a multiplex one.

For optimal results, Stilla Technologies recommends the use of high-quality quenchers such as Black Hole Quencher® (BHQ®). These quenchers absorb broadly, do not emit light, and thus allow the use of multiple reporters with the same quencher (see Table 2 for compatible fluorophores).

Here are some guidelines for assay design:

- Recommended primer and probe concentrations on the naica® system are 0.125 to 1 µM.
- Before multiplexing, it is recommended to test each primer pair and the corresponding probe in simplex reactions to verify PCR amplification.
- Increasing the concentration of probes increases the basal fluorescence signal of the negative droplets in the corresponding detection channel, and to a lesser extent in the adjacent channels due to spill-over.
- When multiplexing, depending on the primer and probe design, competition between the different sequences can occur (for example, primer heterodimer formation or non-specific annealing). High concentrations of primers and probes can aggravate these phenomena. Thus, when combining several assays for high multiplexing, it is recommended to start with low concentrations of primers for all assays (e.g., 0.25 µM), and increase the concentrations gradually up to 1 µM if needed (for example to increase amplification efficiency). Note: When optimizing primer and probe concentrations for a given target, it can be useful to determine the limiting factor first. For example, if the primers are the limiting factor, it is useless to increase probe concentrations.

Other important aspects to consider for assay optimization:

- When designing primers and probes, try to homogenize the melting temperatures of all the primers together and all the probes together. This will make it easier to pinpoint the optimal elongation temperature for all targets.
- When designing probes, it is recommended to use as short a sequence as possible, i.e. <20 nucleotides, to place the fluorophore and quencher in close proximity and result in more efficient quenching. The result is increased fluorescence differences between positive droplets (where probes are hydrolyzed) and negative droplets (where probes remain intact).

5.2 Non-specific fluorescent intercalating dye: EvaGreen®

When setting up an experiment using EvaGreen®, it is important to remember that high concentrations of any dsDNA initially present in the PCR mix will lead to higher basal fluorescence. Moreover, although EvaGreen® has a higher affinity for dsDNA, the dye will also be able to bind with lower affinity to any oligonucleotides present, thus also contributing to basal fluorescence.

For optimal estimation of the sample concentration, use 0.2 up to 1,000 copies of template/μL of final reaction.

Depending on the type of DNA to quantify and the intrinsic properties of the assay, it may be necessary to assess the practical dynamic range.

6 How to perform Crystal Digital PCR® on the Geode

6.1 The Geode program steps for Crystal Digital PCR®

To perform Crystal Digital PCR® three elements must be included in the Geode program:

- a partitioning step (droplet generation)
- thermal cycling steps (PCR amplification),
- a release step (pressure decrease and stabilization of temperature).

Note:

- The operating input pressure of the Geode should be maintained between $1,220 \pm 20$ mbars (via the compressed air system (ISPTLAPG3) delivered with the naica® system) (see Figure 9).
- Stilla Technologies recommends that users start from template programs pre-loaded on the Geode to create new custom programs.
-

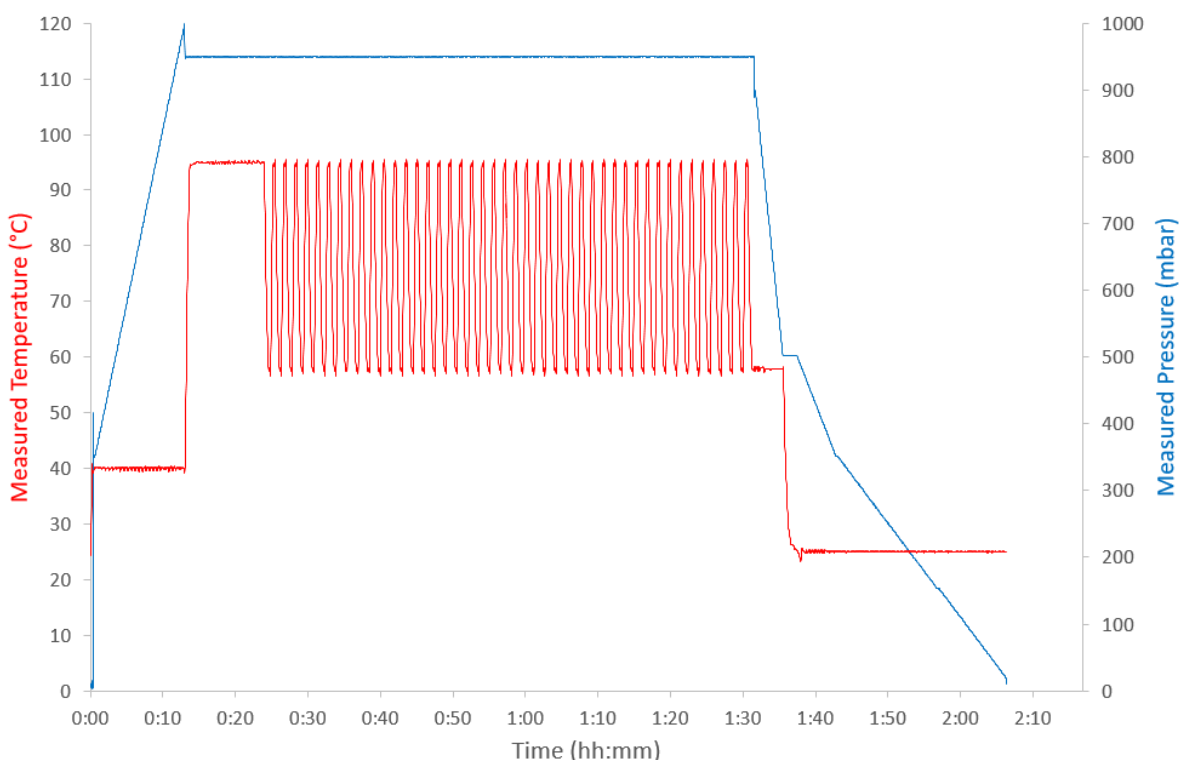


Figure 4: Typical graph showing the evolution of the measured pressure and temperature during the run of a default program for Sapphire Chip loaded on the Geode named “Template Stilla PCR 45 cycles”.

6.1.1 Partitioning

Partitioning is always the first step of a Crystal Digital PCR® program.

For optimal Crystal Digital PCR® the partitioning temperature must be set at:

- 40°C for Sapphire Chip
- 25°C for Ruby Chip

If a modification of the partitioning temperature is required, please contact Stilla Technologies before proceeding.

Default pressure settings to enable droplet generation:

- Sapphire Chip
Pressure increases from atmospheric pressure (AP) to AP + 1000 mbar (followed by a drop to AP + 950 mbar at the end of the partitioning step).
- Ruby Chip
Pressure increases from atmospheric pressure (AP) to AP + 750 mbar (followed by a drop to AP + 700 mbar at the end of the partitioning step).

For Sapphire Chip, the generated droplets have an average volume of ~0.62 nL when using the naica® PCR MIX and the naica® multiplex PCR MIX.

For Ruby Chip, the generated droplets have an average volume of ~0.22 nL when using the naica® PCR MIX and the naica® multiplex PCR MIX.

6.1.2 Thermal Cycling

During the PCR amplification phase, the pressure is fixed to ensure optimal Crystal Digital PCR® performance.

- Sapphire Chip: AP + 950 mbar
- Ruby Chip: AP + 700 mbar

If a modification is required, please contact Stilla Technologies before proceeding.

6.1.3 Pressure release

The pressure release steps consists of the return of temperature and pressure conditions to the ambient state. It should always be the last step of the Crystal Digital PCR® Geode program.

Note that the pressure release step is different for Sapphire and Ruby chips.

6.2 Template programs for Crystal Digital PCR®

Five template programs recommended by Stilla Technologies are provided on the Geode by default (Table 4 to Table 8)

- The “Sapphire FAST-Template PCR 45 cycles.js” program to perform a Fast PCR experiment for Sapphire Chip.
- The “Sapphire Template PCR 45 cycles.js” program to perform a PCR experiment for Sapphire Chip.
- The “Sapphire Template RT-PCR 45 cycles.js” program to perform an RT-PCR experiment for Sapphire Chip.
- The “Template Ruby PCR 45 cycles” program to perform a PCR experiment for Ruby Chip.
- The Template Ruby RT-PCR 45 cycles to perform an RT-PCR experiment for Ruby Chip.

Stilla Technologies recommends the use of the following cycling parameters for PCR and RT-PCR experiments in Sapphire Chips (Table 4 to Table 6):

Table 4: Crystal Digital PCR® template called “Sapphire FAST-Template PCR 45 cycles” using the recommended naica® PCR MIX reagent (EvaGreen® dye) with Sapphire Chip. The total duration of the default program is approximately 2 hours.

STEP	TEMPERATURE (°C)	PRESSURE * (mbar)	DURATION **
Partition	40*	AP to +950	12 min
Initial denaturation	95	+950	3min
PCR (45 cycles)	95	+950	10 sec
	55-65	+950	15 sec
Release	Down to 25	Down to AP	33 min

*Should not be modified, ** Duration states indicative times, variances may occur.

Table 5: Crystal Digital PCR® template called “Sapphire Template PCR 45 cycles” using the recommended naica® multiplex PCR MIX (Fluorescently labeled probes) with Sapphire Chip. The total duration of the default program is approximately 2 hours and 30 min.

STEP	TEMPERATURE (°C)	PRESSURE * (mbar)	DURATION **
Partition	40*	AP to +950	12 min
Initial denaturation	95	+950	10 min
PCR (45 cycles)	95	+950	30 sec
	55-65	+950	15 sec
Release	Down to 25	Down to AP	33 min

*Should not be modified, ** Duration states indicative times, variances may occur.

Note: An initial 10-minute denaturation time at 95°C is not strictly required for the naica® mastermixes; three minutes is sufficient for the hot-start enzyme included in these mixes.

Table 6: Crystal Digital PCR template for RT-PCR application called “Sapphire Template RT-PCR 45 cycles” with Quanta Biosciences qScript™ XLT One-Step RT-qPCR ToughMix® with Sapphire Chip. The total duration of the default program is approximately 2 hours and 30 min.

STEP	TEMPERATURE (°C)	PRESSURE * (mbar)	DURATION **
Partition	40*	AP to +950	12 min
cDNA synthesis	50	+950	10 min
Initial denaturation	95	+950	1 min
PCR (45 cycles)	95	+950	30 sec
	55-65	+950	15 sec
Release	Down to 25	Down to AP	33 min

*Should not be modified, ** Duration states indicative times, variances may occur.

Stilla Technologies recommends the use of the following cycling parameters for PCR and RT-PCR experiments in Ruby Chips (Table 7 and Table 8):

Table 7: Crystal Digital PCR® template called “Template Ruby PCR 45 cycles” using naica® PCR reagents (naica® PCR MIX, naica® multiplex PCR MIX) with Ruby Chip. The total duration of the default program is approximately 2 hours.

STEP	TEMPERATURE (°C)	PRESSURE * (mbar)	DURATION **
Partition	25*	AP to +750	40 min
Initial denaturation	95	+700	3min
PCR (45 cycles)	95	+700	10 sec
	55-65	+700	15 sec
Release	Down to 25	Down to AP	2 min

*Should not be modified, ** Duration states indicative times, variances may occur.

Table 8: Crystal Digital PCR template for RT-PCR application called “Template Ruby RT-PCR 45 cycles” with Quanta Biosciences qScript™ XLT One-Step RT-qPCR ToughMix® with Ruby Chip. The total duration of the default program is approximately 2 hours and 20 min.

STEP	TEMPERATURE (°C)	PRESSURE * (mbar)	DURATION **
Partition	25*	AP to +750	40 min
cDNA synthesis	50	+700	10 min
Initial denaturation	95	+700	1 min
PCR (45 cycles)	95	+700	30 sec
	55-65	+700	15 sec
Release	Down to 25	Down to AP	2 min

*Should not be modified, ** Duration states indicative times, variances may occur.

In addition to the listed PCR and RT-PCR experiment Geode programs, further Geode programs dedicated to commercial kits from Stilla Technologies are available.

The Geode program for the naica® IQ/OQ Kit is pre-loaded on the Geode in the template directory for Sapphire Chip.

Other kit-specific programs are not pre-loaded by default on the Geode, but can be downloaded from the Stilla Technologies website under Technical Resources and imported onto the Geode. Refer to the section “Import or export a program” of this manual for instructions to import a program to the Geode.

For Crystal Digital PCR® experiments using kit-specific programs, please follow the specifications provided with the commercial kit IFU.

Notes:

- The above-described templates for PCR and RT-PCR programs are already pre-loaded in the Geode. They should be used as starting templates to create new assay-specific custom programs.
- When creating a custom program, the user should always consider the operational specifications of the thermal block.
- The total duration of a Crystal Digital PCR® program varies with the temperature ramp rate of the thermal plate, and the number of cycles during PCR amplification.
- The same Crystal Digital PCR® program used on two different instruments may have slightly different durations. This is due to small intrinsic variations of the thermal plate from one Geode to the other.

6.3 Additional protocols on demand

Stilla Technologies continuously develops new protocols to support the optimal utilization of the variety of naica® system applications.

*All user documentation is accessible on the Technical Resources webpage:
<https://www.stillatechnologies.com/technical-resources/>*

The Geode software is exclusively updated by a Stilla Technologist Service Specialist.

6.4 Performing a Crystal Digital PCR® run on the Geode

6.4.1 Loading chips in the Geode for Crystal Digital PCR®

For detailed instructions for loading the samples into the Sapphire Chip and Ruby Chip, please refer to their respective Instructions for Use and the Quick User Guides.

Note: To mitigate the risk for accumulation of dust particles or residual reagent or oil traces on important surfaces within the Geode instrument, Stilla Technologies recommends cleaning the Geode surface (thermal plate, gaskets) prior to any chip placement in the Geode.

To clean the Geode surface, wet a Precision Wipe with water or a decontamination reagent (such as RNase away) and clean the Geode surface areas. Please refer to [Section III. Material & Equipment](#) for information about the recommended Precision Wipes.

Do not use the ACL Staticide® anti-static wipes for the described routine thermal plate surface cleaning.

Refer to the Maintenance and Technical support section of this manual for more details about the cleaning and decontamination procedures.

1. Power on the Geode using the switch located at the rear of the instrument (

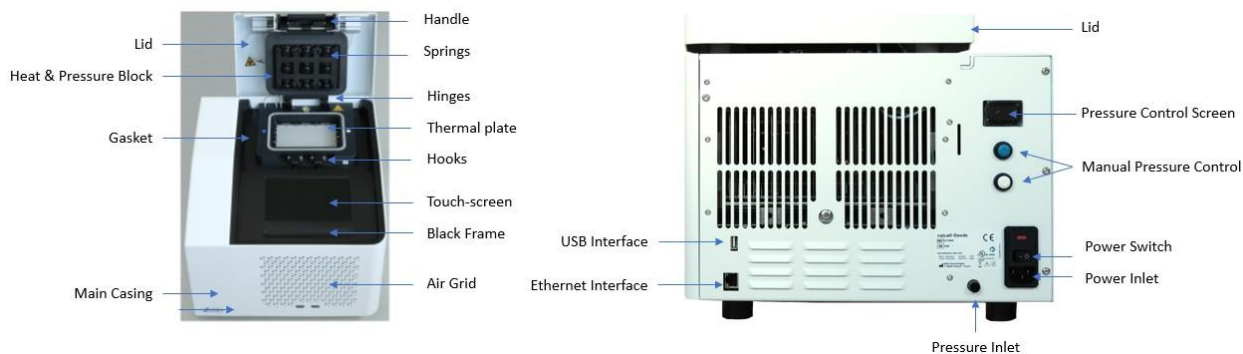


Figure 3) and wait for initialization.

2. Check that the pressure source is connected to the pressure inlet behind the device (

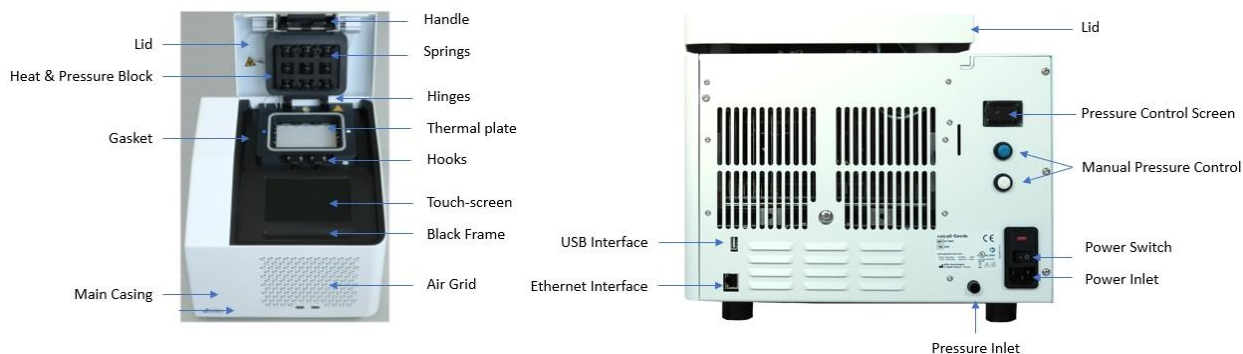


Figure 3). The pressure tube coming from the pressure source (compressed air system)

can be connected to the instrument by simply pushing it into the pressure inlet until it stops.

3. Switch on the pressure source, wait for a few seconds for the pressure to stabilize. Looking at the Geode display, check that the input pressure is between $1,220 \pm 20$ mbar (Figure 9). Adjust the pressure source if needed.
4. Open the lid of the Geode by pulling the black handle downward (Figure 7). Check that the thermal plate is empty and clean. Make sure the gasket around the thermal plate of the Geode is in good working order, and that the thermal plate is kept free of dust particles or moisture.
5. At installation, the Sapphire Chip adaptors and the Sapphire Chip magnetic frame are in place, by default. To change the Geode configuration to Ruby Chip operation, place the provided magnetic adapters next to the existing clamps and make sure they are secured (Figure 5).



Figure 5: Magnetic Ruby Chip clip-on cushions can be easily clipped next to the existing pressure points.

Next, ensure the respective Ruby Chip magnetic frame is placed on the thermal plate with the cut corner placed in the bottom left position.

Place the respective chips in position using the respective magnetic chip frame on the thermal plate. The correct orientation of the respective magnetic frames is supported by a blue (left) and white (white) positioning indicator, both on the magnetic frames as well as on the Geode instrument thermal plate framing (Figure 4).

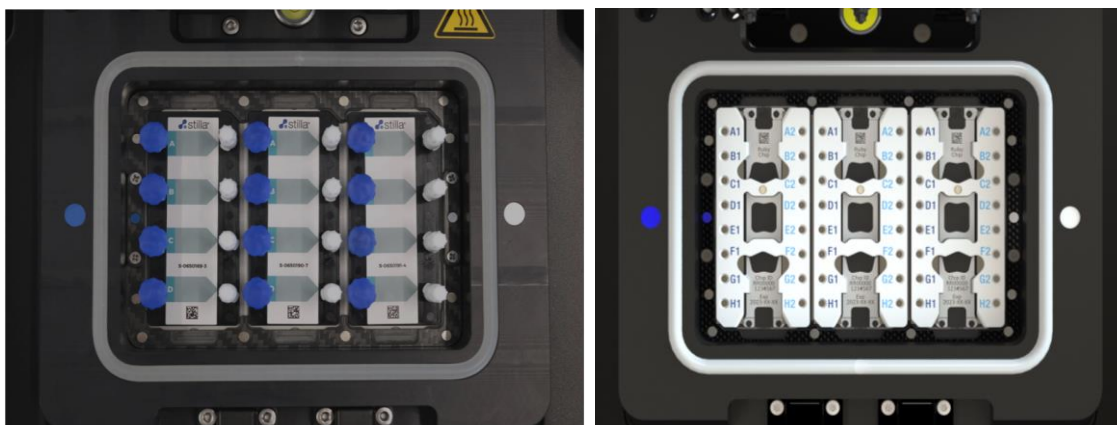


Figure 6: Positioning of the Sapphire Chip (left), and Ruby Chip (right) in the respective magnetic frame on the thermal plate of the Geode instrument.

6. Close the lid of the Geode by pulling the black handle upward (Figure 7). Ensure that the gasket remains in place, ensuring a hermetic seal, while closing the Geode lid.



Figure 7: Opening/closing the lid of the Geode.

When operating Sapphire Chip – please note: It is recommended to load the chips just before starting the Crystal Digital PCR® run on the Geode instrument. Refer to the respective IFU for the Sapphire Chip for detailed instructions on Sapphire Chip operation for Crystal Digital PCR®.

When operating Ruby Chip – please note: The maximum time spent between sample loading of the first Ruby Chip to starting the Crystal Digital PCR® run on the Geode instrument shall not exceed 8 hours. While this maximum time between sample loading and processing is given for optimal use of the Ruby Chip, the results will also depend heavily on the stability of the specific samples to analyze. For sensitive samples, it is recommended to load the chips just before starting the Crystal Digital PCR® run on the Geode instrument. To ensure the correct positioning of the Ruby Chip in the Geode instrument, it is recommended to place them one by one, positioning the bottom of the chip first and then laying the chip flat on the thermal block (Figure 8: Positioning the Ruby Chip in the Geode, starting by the bottom of the chip.). Refer to the Ruby Chip IFU for detailed instructions on Ruby Chip operation for Crystal Digital PCR®.

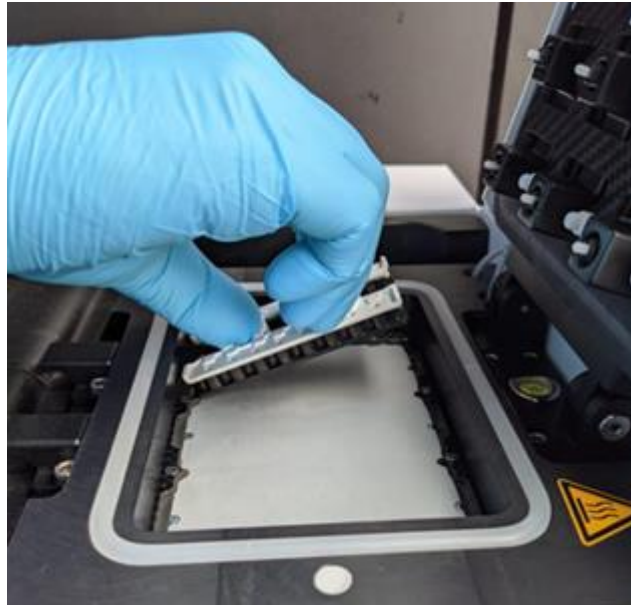








Figure 8: Positioning the Ruby Chip in the Geode, starting by the bottom of the chip.

6.4.2 Starting a run on the Geode

Once the chips are loaded in the Geode, to start a run, the following steps should be followed:
User management settings

1. In the main panel on the screen of the Geode, press “Run” , then “Open” . In the “Open program” window.
2. Select the cycling program in the “local/scripts” or “/templates” sub-folder.
3. Then, press “Open”  and “play”  to start the program.
4. Visualize the graphical progression of the partitioning and thermal cycling programs by clicking on “Live Logs”  accessible from the main panel.
5. Click on “Run”  to go back to check the estimate remaining time.
6. When the Crystal Digital PCR® has ended, a pop-up window is displayed on the Geode screen “PCR successfully completed, touch the screen to continue”.

Notes:

- The remaining time of a program is indicative and relies on the effective temperature ramp rate of the thermal plate and the duration of the stabilization phase at each step. Thus, it can be slightly overestimated by the Geode.

6.4.3 Removing the chips from the Geode

1. Once the run is finished, open the Geode lid.
2. Remove the chips from the respective magnetic frame and close the Geode lid.
 - Hold the Sapphire Chip by the tall white vented PCR caps.
 - Hold the Ruby Chip by the top cover handles in the middle of the Ruby Chip.
3. Place the chips on the respective chip transport tray or a Petri dish containing a Precision Wipe for transport to the Prism3 instrument.
4. Switch off the Geode instrument and the pressure source after the run or, if multiple runs are performed, at the end of each day.


CAUTION!

Please do not try to open the Geode lid during a run. Opening during operation may result in injuries caused by heated materials and/or projections caused by elevated pressure. For your safety, the lid is equipped with a locking security mechanism that prevents opening when the pressure is above 900 mbar (for information on how to pause or abort a run please refer to [How to use the Geode Software User Interface](#))

7 How to use the Geode Software User Interface

7.1 Switch on/off the instrument

To switch on: press the black power button located at the rear of the instrument and wait for initialization.

To switch off: press the “power icon”  (upper right corner) on the front screen and wait 15 sec (the screen should freeze). Then, press the black power button located at the rear of the instrument.

7.2 User interface overview

Users can navigate through the Geode using the tactile graphical interface. The main panel, on the right side of the screen, is composed of 5 menus that can be entered into by simply touching the front screen (**Error! Reference source not found.**Figure 9):

- **“Run”**: to start a selected Crystal Digital PCR® program
- **“Programs”**: to create a new Crystal Digital PCR® program or modify a pre-existing one
- **“Live Logs”**: to visualize the current run in-progress; export the “Live Logs” of the last run program
- **“Reports”**: to view and export reports
- **“System”**: to configure the Geode; export the system logs; access the “About” menu; access the user management settings

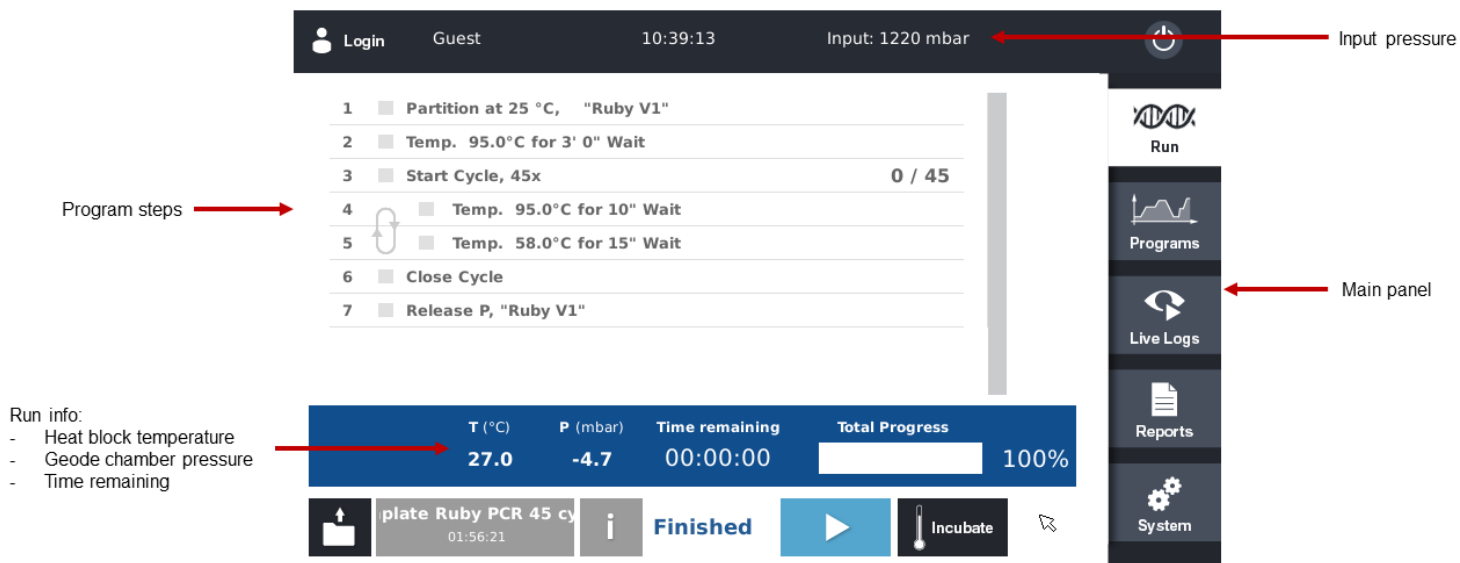


Figure 9: The main panel of the Geode display.

7.3 User management settings

For users operating the Geode in a GMP-regulated environment, the Geode Software v3.3.0 can be configured with three default user groups.

Note:

- Only Stilla Technologies Service Specialists can configure the Geode software v3.3.0 with user groups.
- Only Geode H15000 models with the software v3.3.0 can be configured with user groups.

In this configuration, each user group has different access levels to the Geode software functionalities. A default user account is also available in each user group. The rights and user accounts for each group are detailed in the Table 9 below:

Table 9: Description of the pre-configured user groups and user accounts

Default user group	Administrators	Operators	Guests
Rights	Create, edit and save programs Start, pause and stop a run Start, pause and stop an incubation View and export live logs View and export run reports Manage user accounts and groups	Start, pause and stop a run Start, pause and stop an incubation View and export live logs View and export run reports	Can only view status of an ongoing run
Default user account	Account name: Administrator Default password: admin	Account name: Operator1 Default password: operator1	Account name: Guest No password

At the Geode start-up, the user will be asked to login. After logging in, the user lands on the main panel (Figure 9) and the access to the different buttons depends on their user group as detailed in Table 9. The default user account, when no user is logged-in, is the guest account that allows no actions to be performed on the Geode.

To switch user or log out, click on the “Login” button on the top-left corner of the panel (see Figure 9). This button is accessible from every tab of the GUI except when a run is ongoing. If one tries to change user during a run, a pop-up window explaining that such an action is impossible displays.

After one minute of inactivity, the Geode will automatically log out the signed in user, and return to the Guest account.

Only users in the Administrators user group can create new user accounts and assign them to one of the three default user groups.

Note:

- *The Geode has been verified and validated only for the default user groups defined in Table 8. Therefore, for optimal Geode functioning, Stilla Technologies does not recommend modifying the rights of default user groups nor defining custom user groups with different combination of rights.*

Only users in the Administrators user group can create modify or create new user groups. To create a new user account, click on the System menu from the main panel. Enter the User Management settings (**Error! Reference source not found.**) by clicking on the User Management button (**Error! Reference source not found.**).

By clicking on the “User Management” button in the “User Management settings” panel, a user from the Administrators user group can add or remove user accounts, define their properties and manage passwords.

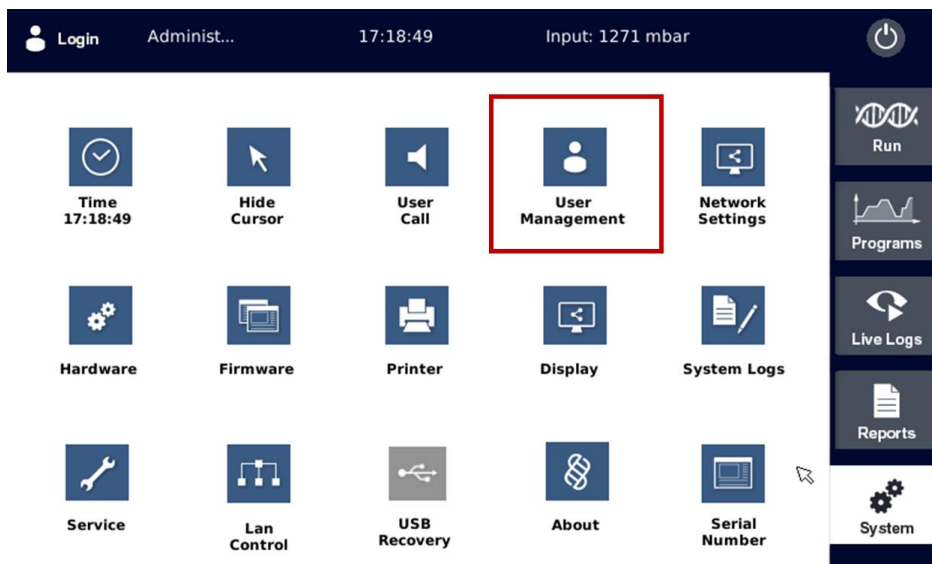


Figure 10: Entering the User Management settings

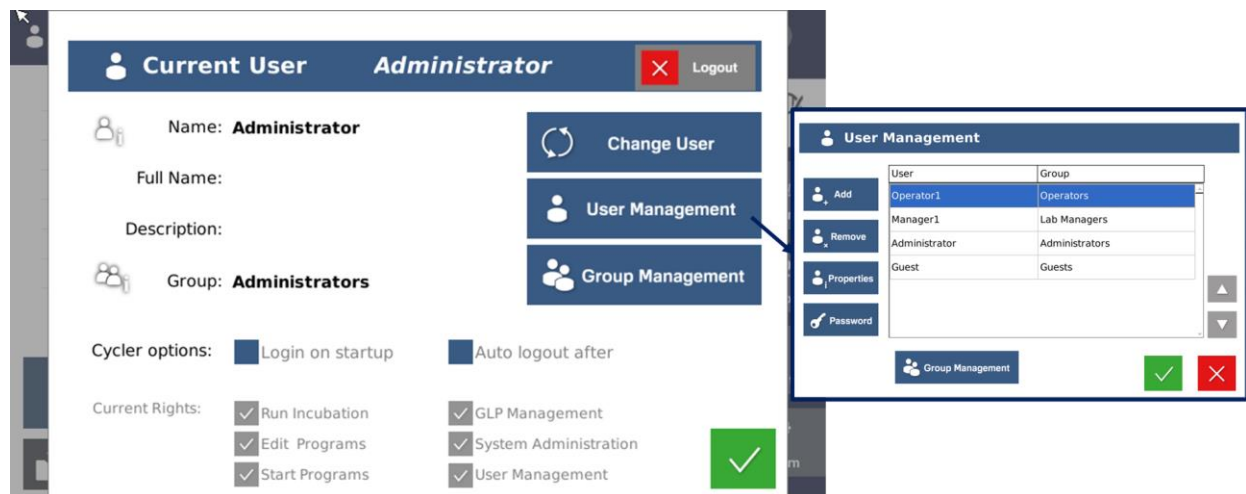


Figure 11: User management settings panel

7.4 Creating a custom program


Note:

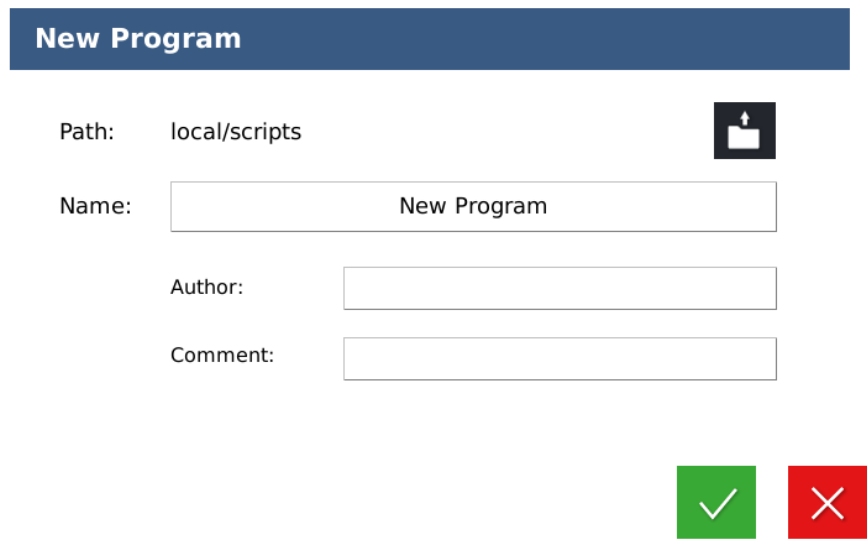
- To create a custom program, Stilla Technologies recommends starting from and editing an existing template program.
- For Geodes configured with the user management feature, program creation and editing are by default only accessible to users from the Administrators user group.
- When creating a custom program, the user should always consider the operational specifications of the thermal block.

7.4.1 Create a new program


To create a new program:

1. In the main panel, press "Programs" and "New Program" (Figure 12). Then, define the name of the program as well as the author, add comments, or change the location for the

program (by default in “local/scripts”). Proceed to creating the program by pressing the “validate icon” .



New Program

Path: local/scripts 

Name:

Author:

Comment:



 

Figure 12: Create a New Program.


2. Configure steps of the new program by choosing from the 5 commands (note: the pressure command should only be used by advanced users). To do this, select the desired command on the left side and choose the position to insert it on the right side in the list, then press the “plus icon”  to insert it (Figure 13). Each available command is detailed in Table 10.

Table 10 – Description of each of the 5 commands available to create and edit programs

COMMAND	DESCRIPTION
Partition 	This command should always be the first step of a Crystal Digital PCR® program. It allows for the droplet generation (select “Sapphire V1” in the drop-down menu for a Sapphire Chip program or “Ruby V1” for a Ruby Chip program).
Temperature 	Use this command to insert a temperature step.
Cycle 	Use this command to define a program cycle (loop), consisting of several individual steps.
Release 	This command should always be the last step of a Crystal Digital PCR® program. It allows to return to ambient temperature and atmospheric pressure conditions (select “Sapphire V1” version in the drop-down menu for a Sapphire Chip program or “Ruby v1” for a Ruby Chip program).
Wait 	Use this command to maintain the temperature defined at the previous step for a given duration.

The first command to be inserted should always be “Partition ” at 40°C for the Sapphire Chip or 25°C for the Ruby Chip, and the last command should always be “Release ” (either Sapphire V1 or Ruby V1).

- Edit the parameters of the steps inserted by pressing the “pencil icon” , or discard an inserted step using the “delete icon” .

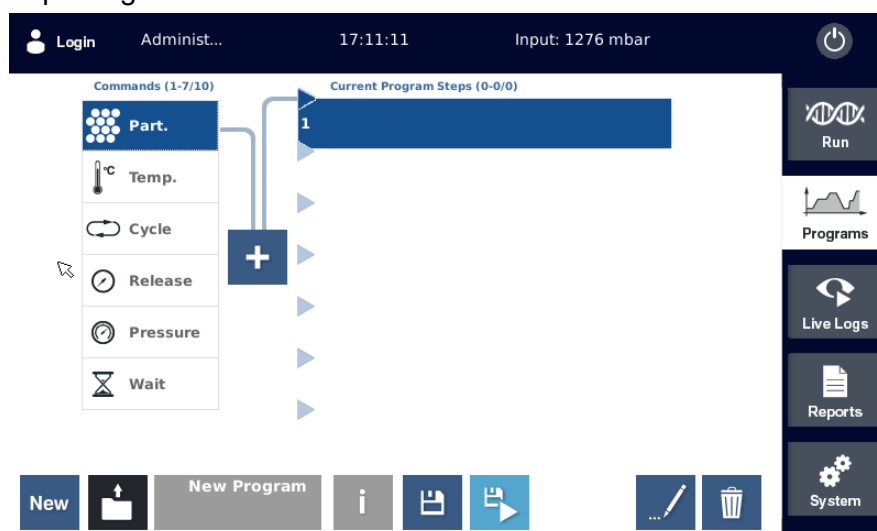








Figure 13: Select the steps of the program.

- Press the “save icon” to save the program, or the “save & play icon” to save and start a program (check that the Geode lid is correctly closed before starting).

7.4.2 Edit an existing program

To edit an existing program:

1. In the main panel, press the “Programs icon”, then the “Open icon” . Select the desired template program (in “local/scripts” or “/templates”) and press “Open”.
2. Use the “plus icon”  or the “delete icon”  to add or delete a command. Select a specific command (e.g. a temperature command) and then press on the “pencil icon”  to modify its parameters.
3. Press the “save icon”  to save the program, or the “save & play icon”  to save and start a program (check that the lid is correctly closed before starting).





All pre-existing programs can be modified by editing:

- the temperature parameters
- the total number of cycles

7.5 Importing or exporting a program

This functionality is only available for users from the Administrators’ group.

Crystal Digital PCR® programs (.js) can be imported from a USB stick to the Geode or exported from the Geode to a USB stick. In both cases:

1. Plug a USB key in the USB port at the front of the instrument.
2. Wait for the pop-up indicating that the USB key has been recognized, then press the “validation icon” .
3. In the main panel, press “Programs”, then the “open icon” .
4. Select the program(s) to be imported (resp. exported) from the USB stick (resp. from the “local/scripts” or “templates” directory of the Geode). To select multiple programs, check the box “multiple selection”.
5. Press on the “copy icon”  on the right side, a new page is displayed.
6. On the new page, press on the “local/scripts” (when importing) or “usb” (when exporting) folder on the right panel. If needed, select a subfolder in the “usb” folder.
7. Press the “validation icon” .

Note :

- For Geodes configured with the user management feature, program importing and exporting is by default only accessible to users from the Administrators user group.

7.6 Run

Note:

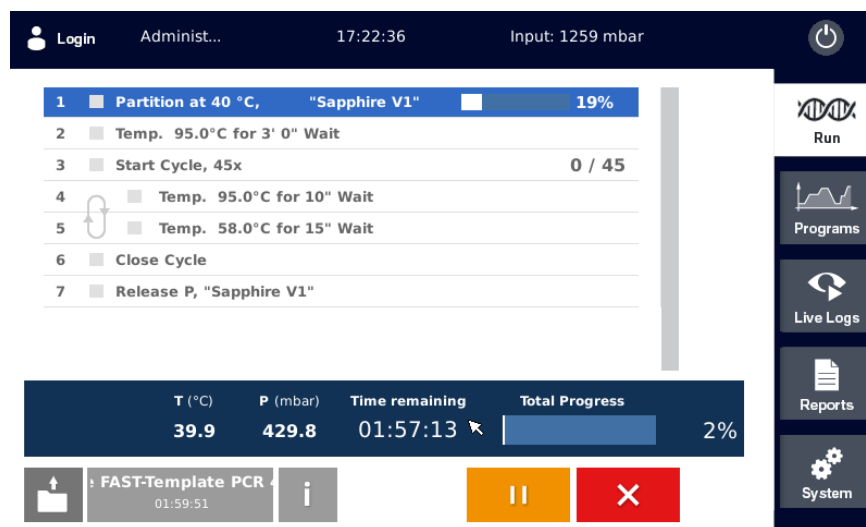


- For Geodes configured with the user management feature, the starting, pausing and stopping a run is by default only accessible to users from the Administrators or Operator group.
1. In the main panel, press “Run 

Figure 14: Run page during program play.

Notes:

- A confirmation popup will appear to confirm if the user wants to start a Ruby Chip or a Sapphire Chip run.
 - A warning popup will appear if the user launches a run starting with the “Partition” command of the Sapphire Chip (resp. Ruby Chip) and ends with the “Release” command of the Ruby Chip (resp. Sapphire Chip).
 - A warning popup will appear if the user launches a run starting with the “Pressurization” command.
 - The internal source pressure of the Geode during the cycling step is preset by Stilla Technologies and this should not be modified by the user, for optimal results. If a modification is required, please contact Technical Support before proceeding.
3. Visualize the temperature profile of the thermal plate and the pressure profile in the Geode chamber during a Crystal Digital PCR® (automatically recorded) by pressing on “Live Logs” in the main panel.

The temperature profile is displayed in red and the pressure in blue (Figure 15). Use the arrows and magnifying buttons to navigate through the diagram. The live record will be complete as soon as the PCR program has ended. It can be exported by plugging in a USB key and pressing the “export icon” .

Further instructions are detailed in section: Export Live Logs or system logs for troubleshooting support

Notes:

- The “Live logs” file of a run is erased from the Geode memory at switch off or when another Geode run is started.
- For Geodes configured with the user management feature, the visualization and export of live logs is by default only accessible to users from the Administrators or Operator group.

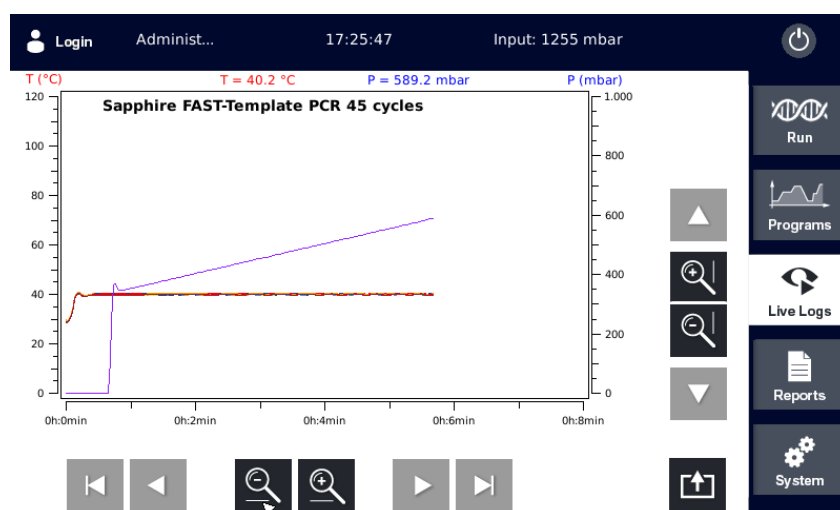




Figure 15: Live graph of measured pressure and temperature during a run.

4. When the run is done, a pop-up window is displayed with the message “PCR completed successfully, touch the screen to continue”. Touch the screen to close it.

7.7 Pause a program

1. During a run, press the “pause icon”  to temporarily interrupt the Crystal Digital PCR®.
2. Confirm the request in the appearing window and the program will be paused.
3. Press the “play icon”  to continue the program.

7.8 Abort a program

During a run, press the “stop icon”  to abort a run. Error! Reference source not found.

If the program is aborted during a Sapphire run, two options are then available:

“Controlled release”: the program proceeds to the pressure release step and lasts several tens of minutes. Once the release is done, the lid can be opened. This option preserves the droplet crystals for Sapphire chips .

“Quick release”: The pressure in the Geode goes straight back to atmospheric pressure as fast as possible without going through the release step. The Geode lid can be opened as soon as the safety locks of the handle opens. This option does not preserve the droplet crystals for Sapphire chips.

If the program is aborted during a Ruby run, only one option is available: “Quick Release”. This option preserves the droplet crystals in Ruby.



Figure 16: Stopping a Sapphire or Ruby Chip run

After aborting an experiment, a Good Laboratory Practice (GLP) report is always generated.

For users from the Administrators and Operators groups, GLP reports are accessible through “Reports” tab in the main panel and can be exported on a USB key (see section Records management).

7.9 Records management for traceability and troubleshooting

7.9.1 Overview of the different record files

After each Geode run, the three following records are generated:


- “Live logs”: records of temperature and pressure measurements throughout the run. This file is erased from the Geode memory at switch off or when another Geode run is started.
- “System logs”: record of system information about the runs started, requests for scripts launch, different requests from the Geode’s data sensors during the runs, error messages during runs. If no software update is implemented, this file is never erased and is populated over time.

- “GLP reports”: GLP stands for “Good Laboratory Practice”. The GLP report contains information about the run such as the name and detail of the PCR program, the user who launched it and a timestamp. Additionally, if an error arises during a run, the error will be logged into the GLP report.

A GLP report is created after each run. The GLP reports cannot be edited nor deleted from the Geode memory.

The GLP report is saved in the Geode’s memory in two different formats:

- a “glp” format that can be opened from the Geode directly
- a “pdf” format that can be exported via a USB stick.

The GLP reports are accessible by clicking on the “Reports” tab of the main panel and then on the “open” icon  (Figure 17). GLP reports are named following the nomenclature “ProgramName_Date_Time” and are listed following alphabetical order (Figure 17, top picture).



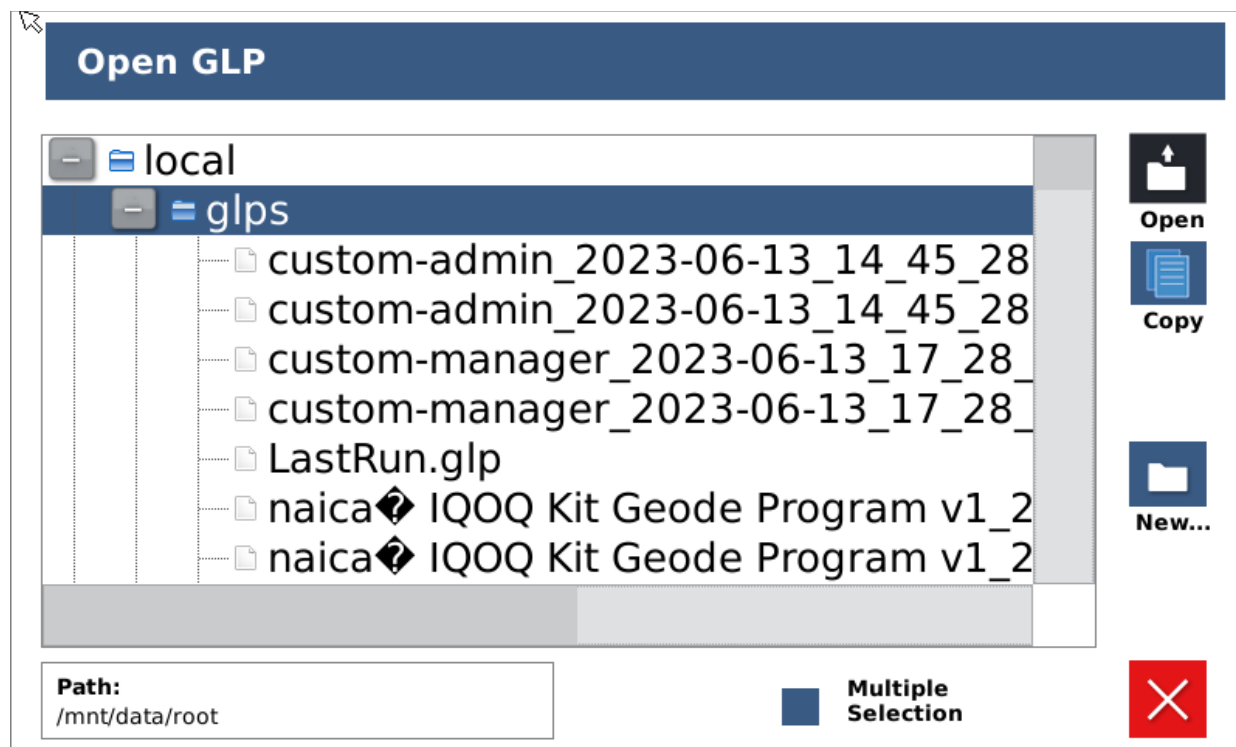


Figure 17: Access to the list of GLP reports (bottom) recorded in the Geode by clicking on the "Reports" menu on the main panel (top).

An example of a GLP report as displayed on the Geode is shown in Figure 18 below.

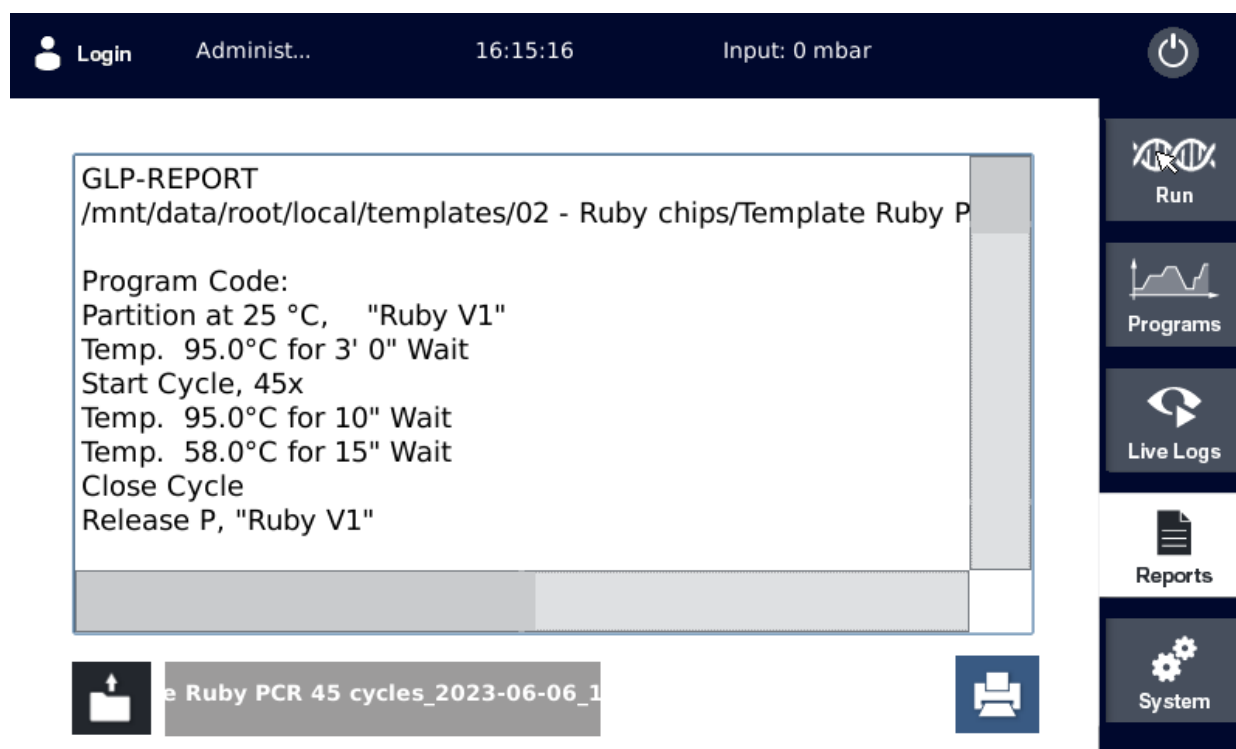





Figure 18: Example of the display of a GLP report on the Geode's screen.




In case of failure during a run, it is advised to export the Livelogs and the System logs to help Stilla Technologies' technical support team troubleshoot the issue.

7.9.2 How to export the Live Logs of the last run program


When the program ends, do not run another program, or shut down the instrument immediately, as this will erase the Live Logs.

1. Plug a USB key in the USB port at the front of the instrument.
2. Wait for the pop-up indicating that the USB key has been recognized, then press the “validation icon” .
3. In the main panel, press “Live Logs”, then the “export icon” .
4. Keep pressing the “validation icon”  until the end.
5. A “Live Log” folder has been created on the USB key. It contains all the information that may be sent to Technical Support for maintenance purposes.

7.9.3 How to export the System Logs

1. Plug a USB key in the USB port at the front of the instrument.
2. Wait for the popup indicating that the USB key has been recognized, then validate .
3. In the main panel, press “System”, then click on “System Logs” .
4. Click on “Send Log-File to USB-Stick”.
5. Always validate  until the end.
6. A “syslog” folder has been created on the USB key. It contains all the information that might be necessary got the Technical Support for maintenance purposes.

7.10 Other functionalities

All other functionalities can be accessed by users from the Administrators’ group by pressing “System” on the main panel. For the user management settings () , refer to the User management settings section. The other functionalities are intended for maintenance purposes only and shall only be modified by a Stilla Technologies Service Specialist or following detailed instructions from a Stilla Technologies Service Specialist.

8 Troubleshooting

The Table 11 lists the recommendations for the indicated Geode display screen messages.

Table 11: Troubleshooting the Geode.

DESCRIPTION OF ERROR MESSAGE	RECOMMENDATIONS
<p>“Power failure” (there was a power outage during the run)</p>	<p>Based on the phase of the run in which the power failure occurs, there might be three different scenarios:</p> <p>1) The power failure occurred right at the beginning of the run. In this case, it could be possible that the droplets have not been generated yet. A scan with the Prism6 could confirm the absence of droplets in the chambers. If this is the case, it is possible to restart the experiment with the same chips.</p> <p>2) The power failure occurred after the run began. The normal pressure outflow from the compressed air system (ISPTLAPG3) to the Geode is interrupted, compromising the crystal structure of the droplets. The experiment would then be considered as failed.</p> <p>3) The power failure occurred at the very end of the run or after the run is completed. The experiment may not be affected. Scan the chips in the Prism6 instrument to confirm.</p>
<p>Screen freeze or abnormal screen (e.g., black screen)</p>	<p>Press the top blue button located behind the instrument.</p> <p>If the screen does not respond or responds partially (e.g., no update of the live logs), extract the live and system logs (refer 'Export Live Logs or system logs for troubleshooting support') then wait for the expected end of the run (to potentially save the experiment if the program is still running correctly).</p> <p>If the screen still does not respond or still responds partially, try to stop the run by clicking on “Stop” to preserve the crystal. If nothing changes, reboot the Geode: switch off the power button, wait a few seconds and switch on the power button.</p> <p>If the problem persists, contact Technical Support.</p>
<p>“Lid cannot be opened”</p>	<p>For safety, the lid is equipped with a locking security mechanism that prevents opening when the pressure is above 900 mbar.</p> <p>In all cases, please wait for the current pressure to be lower than 20 mbar and the current temperature lower than 40 degrees before opening the lid.</p> <p>If the waiting time is longer than 10 min after the program ends, deactivate the input pressure and shut down the instrument (turn off the switch at the rear of the Geode).</p>

<p>“There is an under pressure” or “an overpressure issue”.</p>	<p>Ensure that the pressure source is activated and if needed, adjust the pressure delivered to the Geode to $1,220 \pm 20$ mbar (check the touchscreen of the device or the screen of the pressure controller behind the device to ensure the accuracy of the pressure delivered to the Geode). Check that the gasket around the thermal plate is correctly placed. If the problem persists but the gasket is in place, extract the live and system logs (refer to ‘Export Live Logs or system logs for troubleshooting support’) and contact Technical Support.</p>
<p>“A pressure issue occurred”</p>	<p>The measured pressure is either higher or lower than the required pressure during the run. Make sure the gasket around the thermal plate of the Geode is in good working order to prevent any pressure leak.</p> <p>If the problem persists, extract the live and system logs (refer to ‘Export Live Logs or system logs for troubleshooting support’) and contact Technical Support.</p>
<p>“A temperature issue occurred”</p>	<p>The measured temperature is either lower or higher than the required temperature during the run. If the experiment is running, stop it (for Sapphire Chip runs, click on “Controlled Release” to preserve the crystal). Make sure that the Geode is placed at 25 cm from any wall or objects, and that room temperature is lower than 25°C.</p> <p>If the problem persists, extract the live and system logs (refer to ‘Export Live Logs or system logs for troubleshooting support’) and contact Technical Support.</p>
<p>Geode screen turns black with white text lines</p>	<p>Reboot the Geode: switch off the power button, wait a few seconds and switch on the power button located at the rear of the instrument.</p> <p>If the problem persists, contact Technical Support.</p>

9 Maintenance and Technical Support

Maintenance operations of the Geode should be executed by a Stilla Technologies Technical Specialist during a visit on-site or by the return of the device to Stilla Technologies premises. Stilla Technologies cannot be held responsible for any intervention or modification done by the user on devices of the naica® system.

Before the intervention, Stilla Technologies will request the user to decontaminate the instrument following instructions detailed in the Decontamination Protocol and to thereafter fill a Decontamination Certificate; both these documents are provided by the Stilla Technologies Service Specialist.

The Geode gasket is an accessory of the Geode instrument to support the air tightness of the instrument. As any gasket in comparable equipments, such gaskets must be changed depending on the frequency the Geode instrument is used.

Make sure the gasket around the thermal plate of the Geode is in good working order, and that all instruments are kept free of dust particles or moisture.

For technical questions or any issue regarding instrument or software malfunction contact us:

Monday to Friday, 9:30 AM - 6:30 PM Central European Time (CET).

Closed on French bank holidays.

Phone: (+33) 9 82 27 47 47

Email: support@stilla.fr.

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

We will try our best to answer as promptly as possible.

Please see below for maintenance-related instructions for the Geode and the compressed air system (ISPTLAPG3).

9.1 Cleaning and decontamination

For optimal performance, it is recommended to limit the contact of dust particles with the naica® system. All naica® system devices should be switched off and power sockets should be unplugged before cleaning and decontamination operations.

Material necessary for the decontamination procedure:

- Gloves;
- Glasses;
- Mask;
- Laboratory coat;
- Hydroalcoholic disinfectant solution for device surfaces, commonly used for biological and medical devices (e.g. Phagospray).
- Decontaminant solution for device surfaces, used for biological and medical devices, specifically targeting nucleases and DNA contamination (e.g. RNase away).
- Standard laboratory paper towels

Notes:

- Bleach is not recommended; if its use is unavoidable, be sure to abundantly rinse with water after the bleach treatment.

- In case of anticipation of using a similar disinfection product due to non-availability of the above specified Phagospray reference, Stilla Technologies recommends to first test another alcohol-based product on a small, less visible surface of the Geode and Compressed air system instruments.

Cleaning/decontaminating the Compressed air system:

Moisten a paper towel with the disinfectant/decontaminant cleaner specified above and pass it through the external surfaces of all the detachable parts and the external part of the instrument, paying attention to avoid the power connector.

Cleaning/decontaminating the Geode:

Remove the tube coming from the pump, from the rear side of the Geode.

Open the lid of the Geode and take away eventual residual chips.

Remove the magnetic frame laying on the thermo-block surface.

On a bench, put several layers of paper towel and place the magnetic frame on top.

Spray the disinfectant/decontaminant cleaner specified above on the whole surface of the magnetic frame, on both sides and leave it for 5 minutes.

With a clean and dry paper towel absorb the liquid in excess from the magnetic frame and rinse it if necessary.

Please take notice that a Geode instrument may include several magnetic frames for the different naica® system chip consumable types. Ensure to perform the above-described procedure for all magnetic frames and for all magnetic adaptor(s) fitting in the internal part of the lid.

Moisten a paper towel with the disinfectant/decontaminant cleaner specified above and pass it through all the surface of the Geode (paying attention to avoid the power connector), the surface of the thermo-block and the internal surface of the lid.

For the detailed procedure for decontamination of the whole 3-color naica® system, please view the Decontamination Protocol available on the Technical Resources webpage.

9.2 Disposal

- Disposal of naica® system equipment

The disposal of the Geode and the pressure source at the end of the product's life should comply with the current legislation, in force in the country of use, regarding electrical and electronic waste.

9.3 Shipment

If shipping the Geode or the compressed air system (ISPTLAPG3) is required, use all the original packaging and cables provided upon reception of the naica® system.

No chips (Sapphire Chip/ Ruby Chip) must be left inside the Geode.

The original double packaging and protective foam should be used for the Geode. If the original packaging material is not available please contact Stilla Technologies.

To prepare the Geode for shipment, proceed carefully as follows:

6. Remove any chip that may remain in the instrument.
7. Switch off the power switch located at the back of the instrument and remove the power supply cable.

8. Switch off the pressure source and remove the pressure tube from the pressure inlet located behind the instrument, by pushing on the ring-shaped push-in connector while pulling on the tube.
9. Repack the instrument into the original packaging in the same way it was delivered; include the power supply cable in the packaging. For detailed packing instructions, please contact Technical Support.

10 Software License information


1. Geode embedded software license

*©2017-2023 Stilla Technologies. All rights reserved.
For Research Use Only. Not for use in diagnostic procedures.*

10.1 Third-party licenses

The software embedded in the Geode uses the following third-party software components:

- libqxt
- Linux Raspberry Pi
- QextSerialPort
- QSerialDevice
- Qt
- qtcopydialog
- Qwt

Feel free to visit their websites. Note that the software version, as well as the license information of all the third-party software components, is also accessible through the Geode user interface, by pressing “System” in the main panel and “About” .

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MKT-00137 Rev.F