

Ruby Chip for the naica[®] system and Nio[®] Digital PCR

Instructions for Use

For Research Use Only.

Not for use in diagnostic procedures.

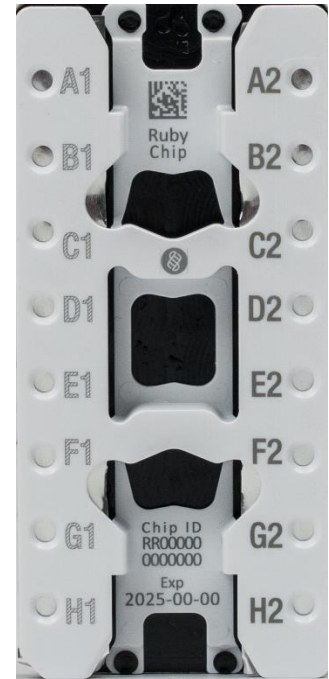
Product Name

Ruby Chip (Ref.C16011)

Resources

Depending on the naica[®] system or the Nio[®] Digital PCR configuration, the technical resources for Instructions for Use for the Ruby Chip are available:

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



Intended Use

The Ruby Chip is a consumable used with the naica system and the Nio Digital PCR to perform Crystal Digital PCR[®]. The Ruby Chip is intended to partition nucleic acid samples and to contain the partitioned samples on the chip for amplification and detection of nucleic acids. Partitioning is performed by generating water-in-oil droplets. The Ruby Chip is intended for use by laboratory personnel trained to perform Crystal Digital PCR using the naica system or the Nio Digital PCR. The Ruby chip is intended to be used for reasearch use only and not for use in diagnostic procedures.

The instructions in the present document are intended for Ruby Chip use at altitudes below 500m. For use at higher altitudes, please contact Technical Support.

Composition and Packaging

Each box contains an inflated thermos-sealed aluminum pouch.

Within this pouch are 5 Chip Plates stacked and wrapped with protective foam:

- Within the upper Chip Plate is a Piercing Tool stored in a plastic bag
- The four bottom Chip Plates each hold 3 Ruby Chips, for a total of 12 Ruby Chips.

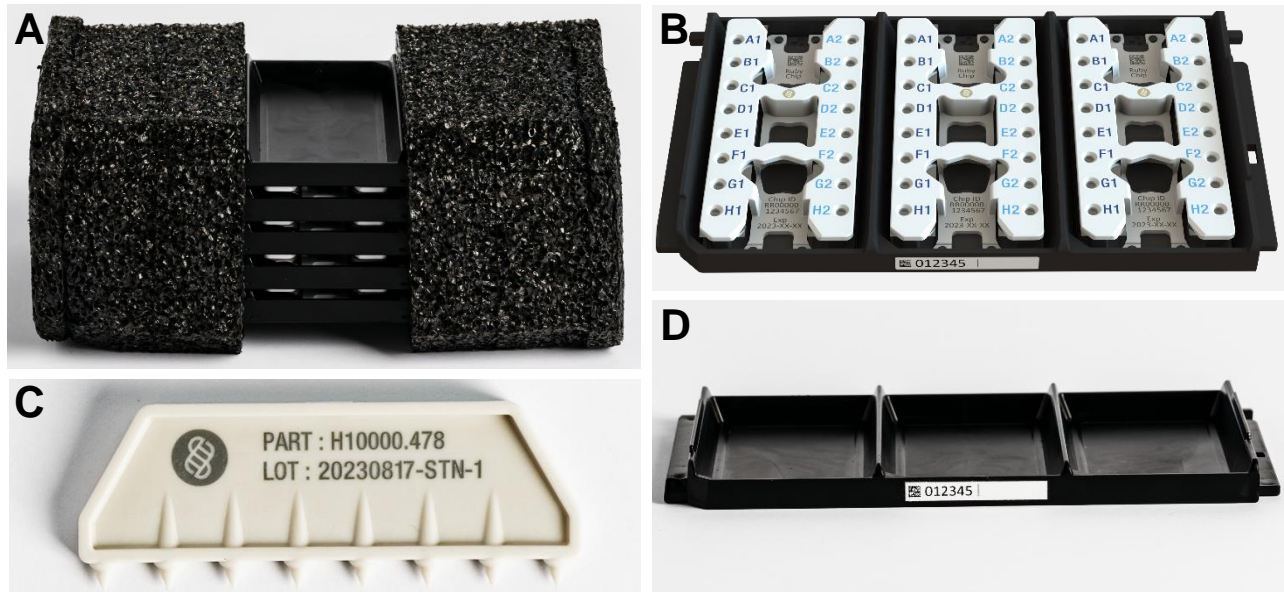


Figure 1 - A) 5 Chip Plates, wrapped in protective foam B) Chip Plate with 3 Ruby Chips
C) Piercing Tool D) Empty Chip Plate

Consumables Required but Not Provided

- Reagents and consumables for nucleic acid purification.
- Standard consumables and equipment for PCR mix preparation
- Assay-specific digital PCR reagents, primers and probes.
- Antistatic wetted wipes (ACL Staticide[®], Reference: SW12)
 - ➔ This product can be ordered from Stilla as a spare part (PN H10000.472) or directly from the supplier Digi-Key using the reference ST1059-ND. [SW12 ACL Staticide Inc | Static Control, ESD, Clean Room Products | DigiKey](#)
- Precision Wipes (Kimtech[™] Science, Reference: 7552, 1 ply, 213x114 mm)
 - ➔ This product can be ordered from standard laboratory suppliers.

Specifications and Performances

General Characteristics

The Ruby Chip is a ready-to-use product, pre-filled with oil. The layout of the Ruby Chip is detailed below (Figure 2):

- The Ruby Chip consists of a total of 16 chambers presented in two columns of 8 chambers.
 - ➔ The individual chambers display the following chamber identifiers:
 - left: A1-H1 in dark blue
 - right: A2-H2 in light blue
- Individual sample mixes are pipetted through the inlet port orifice of each chamber, by piercing through the aluminum foil cover.
- Central handles allow for easy chip transport and handling.
- Each Ruby Chip is identified with a unique serial number (Chip ID) engraved on the chip and embedded in the Chip ID barcode. The Chip ID barcode can be scanned using a barcode reader.
- The expiration date of the Ruby Chip is engraved on the chip.

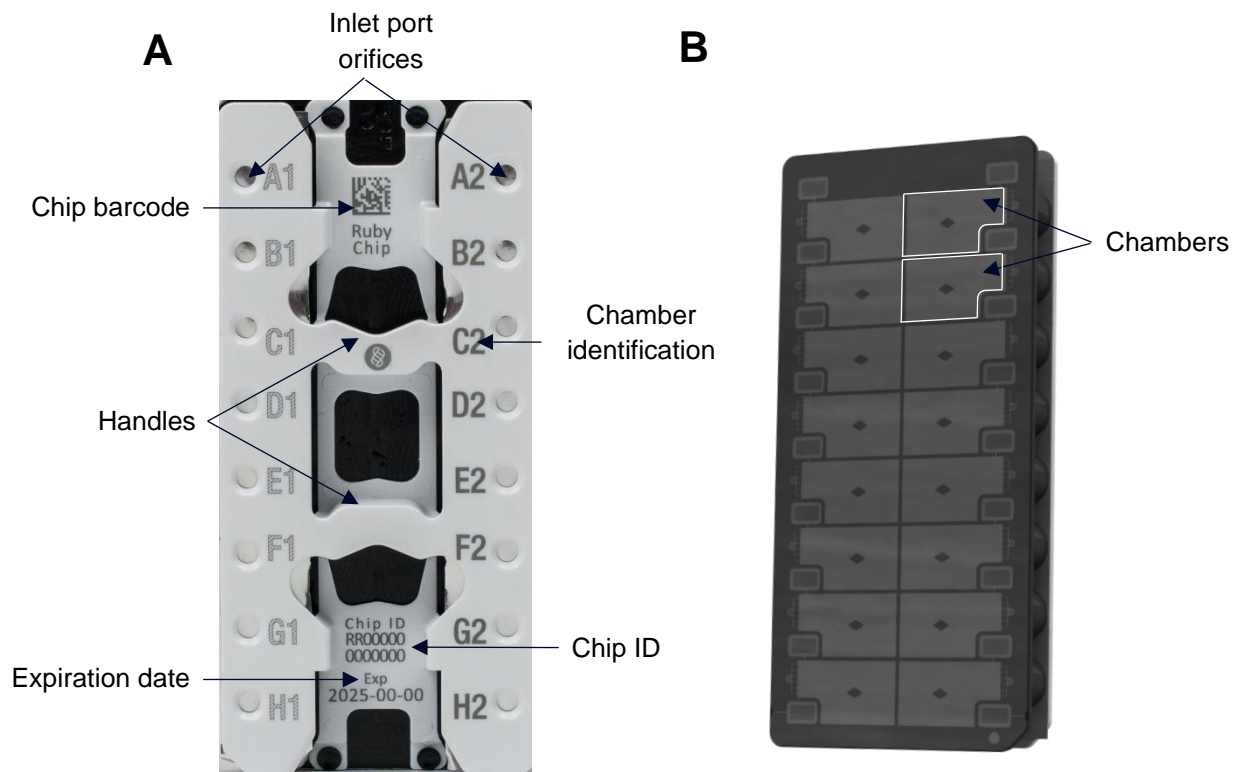


Figure 2: A) Top view of the Ruby Chip B) Bottom view of the Ruby Chip

Table 1: General characteristics

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

General characteristics Ruby Chip	
Capacity	16 samples/chip
Input volume	5 µL/chamber
Droplets per sample	10,000 – 17,000**
Average droplet volume	~ 0.22** nL
Dynamic range*	~ 4.7 log
LOD min (95%)*	down to 0.80**cp/µL
LOD max (95%)*	up to 39,000**cp/µL
Altitude	Operating at max. 1000m above sea level. This document covers the use up to 500m only..
Nio™ Digital PCR compatibility	Nio™+ (PN N30027.0) Nio™ (PN N30027.1) Nio™ E (PN N30027.2)
naica system compatibility	Geode (PN H13000-H15000) Prism3 (PN H22000) Prism6 (PN H24000.6)

(*) Analytical specifications are assay dependent/Values presented here are theoretical and are calculated based on the droplet size, droplet volume and numbers.

(**) Results obtained with naica multiplex PCR MIX and naica PCR MIX.

The Ruby Chip has been fully verified and validated, for optimal technical Crystal Digital PCR performance compatibility, for the following master mixes:

Table 2: Master mixes compatible with the Ruby Chip

Designation	Supplier	Reference
naica multiplex PCR MIX 10X	Stilla Technologies	R10104, R10105
naica multiplex PCR MIX 5X	Stilla Technologies	R10054, R10055
naica PCR MIX 10X	Stilla Technologies	R10106
naica PCR MIX 5X	Stilla Technologies	R10056
qScript XLT 1-Step RT-qPCR ToughMix®	Quantabio	95132

Caution: The intended input material for the Crystal Digital PCR workflow is extracted nucleic acids. The respective extraction purity of the crude sample pre-processing may introduce inhibition effects to the Crystal Digital PCR workflow. It is recommended to perform validation tests to select a compatible extraction protocol for optimal performance of Crystal Digital PCR.

Handling and Storage Upon Reception

- Make sure the Ruby Chip packaging is not damaged upon arrival:
 - If the inflated pouch containing the Ruby Chips is pierced or deflated, do not use the product, and contact Technical Support.
 - If the Chip Plates appear damaged, do not use the product and contact Technical Support.
 - If the Ruby Chip box is delivered upside down, the chips are still usable if the packaging (pouch included) is not damaged. In this case, turn the box in the orientation indicated by the arrows printed on it.
- Keep the Ruby Chip box in a clean area, at room temperature (between +15°C and +25°C), under standard humidity conditions (20-55% in controlled laboratories).
- Store unopened Ruby Chips boxes in the orientation indicated by the arrows printed on the Ruby Chip box.
- Store the Ruby Chips in a well-ventilated place, away from heat, strong bases, strong alkali, and oxidizing agents.
- Do not use the Ruby Chip after the expiration date specified on the box and on the chips.

Opening the Packaging and Storage Once Opened

Inflated Pouch Opening Procedure:

- Keep the Ruby Chip boxes in the orientation indicated by the arrows printed on the Ruby Chip box.
- Remove the pouch from the box and keep it in the orientation indicated by the label (**Figure 3**). Use scissors to cut open the inflated pouch.



Figure 3: Ruby Chip packaging

Chip Plates Opening Procedure:

- Remove the protective foam from around the five Chip Plates.
- Remove the empty top Chip Plate (spare Chip Plate) to access the individual Chip Plates containing the Ruby Chips.

After Opening the Inflated Pouch:

- Store the Ruby Chips in their Chip Plates, inside the box. The inflated pouch and the protective foam can be discarded once opened.
- Keep the Chip Plates upright.



Figure 4: Ruby Chip consumables inside the Chip plate.

Handling Ruby Chips post-opening

- Handle the Ruby Chips with caution.
- Always pick up the Ruby Chip using the handles. Do not touch the underside of the Ruby Chip, as this may compromise further analysis.
- Always keep the Ruby Chip with the chamber inlet ports pointing upwards to avoid oil leakage (**Figure 4**).
- Keep the Ruby Chips in the Chip Plates when loading the chips, transporting them from one step of the workflow process to the next, as well as for storing them after Crystal Digital PCR:
 - For the naica system, when transporting the chips to the Geode, and from the Geode to the Prism3 or Prism6.
 - For the Nio Digital PCR: the Ruby Chips must be in the Chip Plate to be loaded into the instrument.

The Chip Plates are made of conductive plastic material. The Chip Plate will protect the Ruby Chips from electrostatic charges and help to prevent any coalescence of the Droplet Crystal.

- If using less than 3 Ruby Chip consumables per run, use the additional Chip Plate provided in every Ruby Chip box to load and transport the chips.
- Chip Plates can be used up to three times. For cleaning instructions, see below in chapter 4.1.
- Each Chip Plate has a label with a unique barcode that can be scanned with a barcode reader. It is possible to manually write on the label with a pen to identify the Chip Plate
- It is essential to wipe the bottom of the Ruby Chip with anti-static wetted wipes (ACL Staticide®, Reference: SW12) to avoid the problem of Droplet Crystal coalescence.
- All the inlet ports must be pierced regardless of the number of chambers loaded. Use the provided Piercing Tool to pierce all the inlet ports of a Ruby Chip before starting the Crystal Digital PCR run.

- The time spent between sample loading in the Ruby Chip and starting of the Crystal Digital PCR run, should not exceed 8 hours.
 - ➔ Note 1: The waiting time inside the instrument must be taken into account in the total time.
 - ➔ Note 2: While this maximum time between sample loading and processing the chips is given for optimal use of the Ruby Chip, the results will also depend heavily on the stability of the specific samples and assays to analyze, as well as of the analytical needs of each application. For sensitive samples or applications, it is recommended to load the chips just before starting the Crystal Digital PCR run.

Sample Preparation: Principle of the Procedure

Prepare > Amplify > Read > Analyze

Loading samples into Ruby Chips for Crystal Digital PCR includes the following steps: anti-static treatment of the bottom side of the chips, piercing of the inlet ports, and pipetting of the sample.

1. Anti-static Treatment

- Take a Chip Plate and place it on the bench (**Figure 5C**). If using less than 3 Ruby Chips, put the required number of chips in the additional Chip Plate provided in every Ruby Chip box to load the chips and leave the remaining chips in their original Chip Plate.
- Open a pouch of the pre-wetted anti-static wipe (ACL Staticide®, Reference: SW12). Wrap the anti-static wipe around a finger, as indicated in **Figure 5A** and thoroughly wipe the underside and the edges of the Ruby Chip to fully coat it with the anti-static treatment.
- Without turning the Ruby Chip upside down, lift the Ruby Chip and visually check that the entire bottom surface of the Ruby Chip has been fully covered with anti-static solution.
- Wrap a piece of Precision Wipe (Kimtech™ Science, Reference: 7552) around a finger, and wipe off excess anti-static product with (**Figure 5B**).
- Place the Ruby Chip back in the Chip Plate and apply the anti-static treatment to the next chip.
 - ➔ One ACL Staticide® anti-static wipe can be used for up to 3 Ruby Chips, if they are all processed directly one after the other.
 - ➔ If the anti-static wipe appears to have dried out, take a new anti-static wipe to prepare each Ruby Chip.
- Keep the chips in the Chip Plate and continue to the pipetting process.

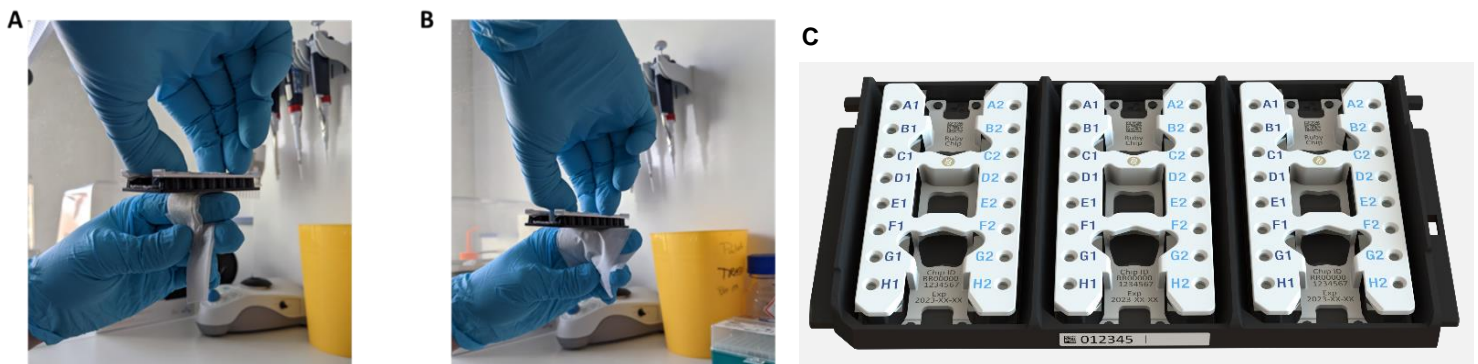


Figure 5: A) Apply ACL Staticide® anti-static solution using pre-wetted wipes.
 B) Wipe off the excess anti-static solution using KimTech™ Precision Wipes.
 C) Keep the Ruby Chip in the Chip Plate.

2. Chip Piercing

- Hold the Piercing Tool in one hand. With the other hand, hold the chip down in the provided Chip plate. Best practice is to place your index or middle finger at the top end of the chip, and your thumb on the bottom end of the chip (**Figure 6**).
- Position the Piercing Tool above a column of wells and in contact with the aluminum foil covering the inlet ports.
- Press the Piercing Tool down to pierce the foil.



Figure 6: Hold the chip with one hand and your Piercing Tool with the other.

3. Sample Mix Pipetting

Note: Stilla Technologies recommends using universal 20 μ L or 10 μ L tips and avoiding using positive displacement pipettes, LTS tips (LiteTouch tip ejection system) or tips covering a large range of volumes (for example 1-100 μ L) for the sample mix injection process. For inquiries about pipette tip compatibility, please contact Technical Support.

3.1 Single-Channel Pipetting

- Load 5 μ L of sample mix in the tip of your pipette.
- Hold the pipette in one hand. and with the other hand, hold the chip down in the provided Chip Plate. Best practice is to place your index or middle finger at the top end of the chip, and your thumb at the bottom end of the chip (**Figure 7**).

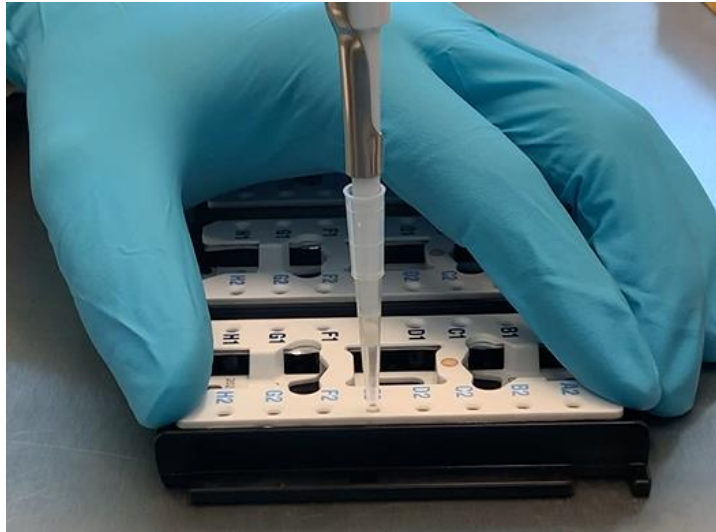


Figure 7: Hold the chip with one hand and your pipette with the other.

- Position the tip of the pipette above the inlet port, holding the pipette vertically above the chip (90° angle with the chip).
- Insert the pipette tip inside the inlet well and touch the bottom of the well with your tip (**Figure 8B**).
- Still keeping your pipette at a 90° angle with the chip, gently lift the pipette tip slightly off of the bottom of the inlet well. The displacement of the pipette tip should be small - approximately 1 mm above the bottom of the inlet well – such that the pipette tip remains inside of the oil (**Figure 8C**).
- Still keeping your pipette steady at a 90° angle with the chip, eject the sample mix by pressing the plunger of the pipette down to the first stop in a continuous and rapid motion. (**Figure 8D**). Do not lift your pipette out of the inlet well as you eject.

Important! Do not press the plunger of the pipette down to the second stop, to prevent the creation of bubbles.

- Remove pipette tip out from the inlet well.

Note: To avoid cross-contamination and the creation of air bubbles, always use a new pipette tip to load each individual Ruby Chip well, even if using the same sample mix for all wells.

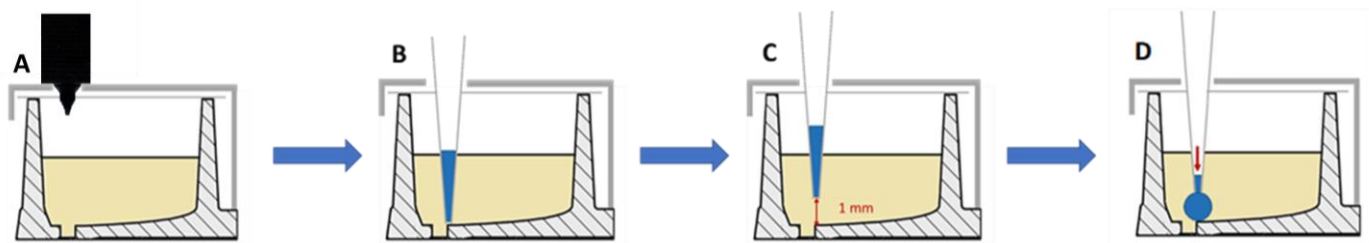


Figure 8: Pipetting process:

- A) Pierce with the pipetting device, B) Reach the bottom of the well with your tip
 C) Stabilize the tip 1mm above, D) Release the mix all at once.

3.2 Multichannel Pipetting (8-channels)

Note: If using a multichannel pipette, ensure that the pipette tips used are compatible with use with a multichannel pipette. For example, if the top part that fits onto the pipette is too thick and rigid, the tips will not make a good seal to the pipette and are therefore incompatible with the multichannel pipette.

- Load 5µL of sample mix in each of the 8 tips of your multichannel pipette.
- Hold the multichannel pipette in one hand, and with the other hand, hold the chip down in the provided Chip Plate. Best practice is to place your index or middle finger at the top end of the chip, and your thumb on the bottom end of the chip (**Figure 7**).
- Position the 8 tips of the multichannel pipette above each of the inlet port, holding the pipette vertically above the chip, at a 90° angle with the chip.
- Insert the 8 pipette tips inside the 8 inlet wells and touch the bottom of the well with your tip (**Figure 8B**).
- Still keeping your multichannel pipette at a 90° angle with the chip, gently lift the pipette tips slightly off of the bottom of the inlet well. The displacement of the pipette tips should be small - approximately 1mm above the bottom of the inlet well – such that the 8 pipette tips remains inside of the oil. (**Figure 8C**). All 8 tips should be at the same height in the chamber inlet ports before injecting (**Figure 9**).
- Still keeping your pipette steady and at a 90° angle with the chip, eject the sample mix by pressing the plunger of the pipette down to the first stop in a continuous and rapid motion. (**Figure 8D**). Do not lift your pipette out of the inlet well as you eject.

Important! Do not press the plunger of the pipette down to the second stop, to prevent the creation of bubbles.

- Remove the multi-channel pipette tips out of the inlet wells.

Note: To avoid cross-contamination and the creation of air bubbles, always use a new pipette tip to load each individual Ruby Chip well, even if using the same sample mix for all wells.

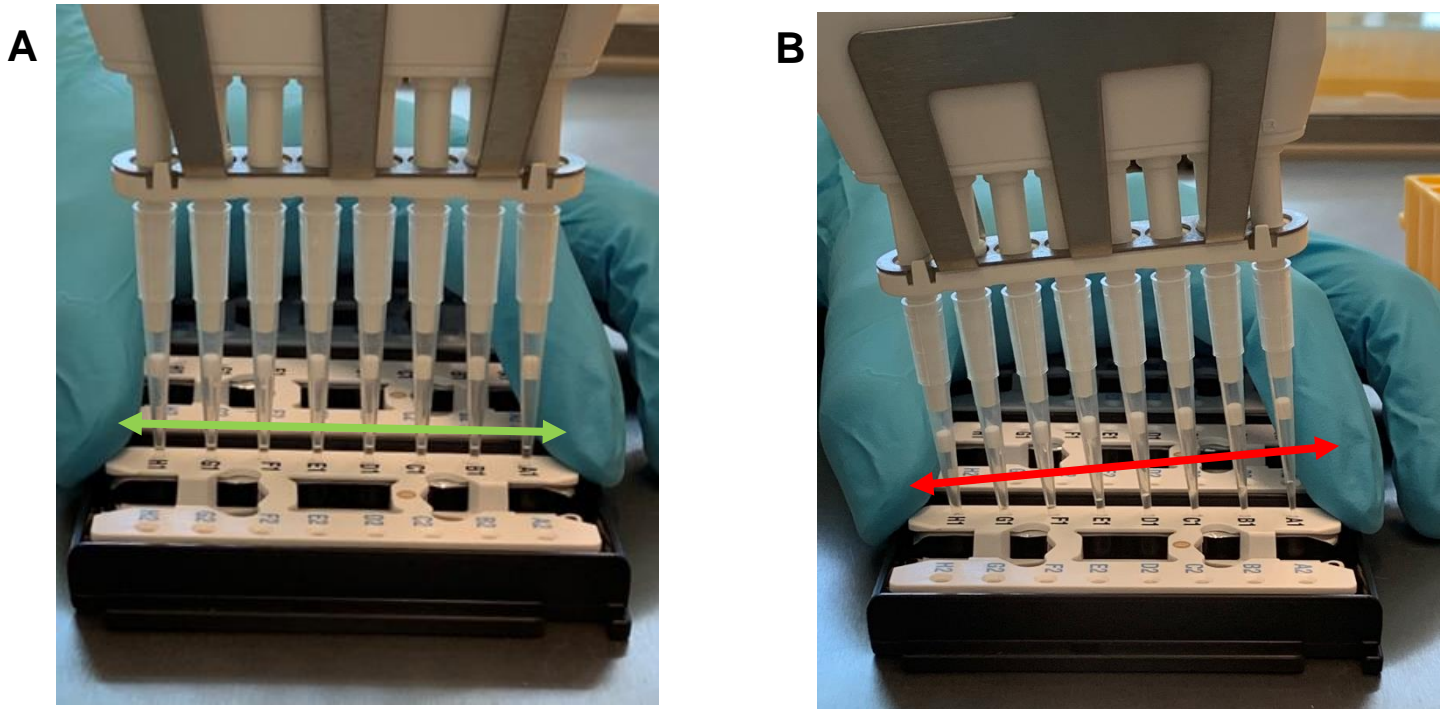


Figure 9: A) Correct positioning of an 8-channel pipette for injection. B) Incorrect positioning.

3.3 Pipetting Robot

The Ruby Chips are also compatible with most pipetting robots. For further information, please refer to the Technical Note for using Ruby Chip with a pipetting robot “Product demonstration – integration of automated liquid handling of Ruby Chip enables a fully integrated workflow solution for high-throughput Crystal Digital PCR”.

4. Reusing or cleaning Chip Plates and Piercing Tools

4.1 Chip Plate Cleaning

Each Chip Plate holds a total of 3 Ruby Chips. As users may want to process fewer than 3 Ruby Chips at once, it is possible to re-use Chip Plates as spares. Chip Plates can be used up to three times if it is cleaned and decontaminated between experiments in order to remove any risks of contamination during experiments.



Figure 10: Chip Plate with only one Ruby Chip

To clean and decontaminate the Chip Plate:

- Remove all used Ruby Chips from the Chip Plate.
- Spray ethanol on a laboratory wipe and wipe the inside as well as the outside of the Chip Plate.
- Spray a decontaminating solution such as RNase away on a laboratory wipe and wipe the inside as well as the outside of the Chip Plate.
- Let the decontaminating solution evaporate. The Chip Plate is ready for additional Crystal Digital PCR experiments.

Note: Additionally, the label on the front of the Chip Plate can be wiped clean with ethanol (it will not erase the number or the QR code) to write on it anew using either a pen or a marker.

4.2 Piercing Tool cleaning

Stilla recommends cleaning the Piercing Tool between each PCR run. Every time the Piercing Tool is to be reused for an experiment, proceed with the following cleaning steps:



Figure 11: Piercing Tool

- Spray decontaminant solution such as RNase away on a laboratory wipe.
- Taking the Piercing Tool in one hand and the laboratory wipe in the other, wipe the row of piercing cones from their bases to their tips. This minimizes the risk of fibers getting stuck on the cones.

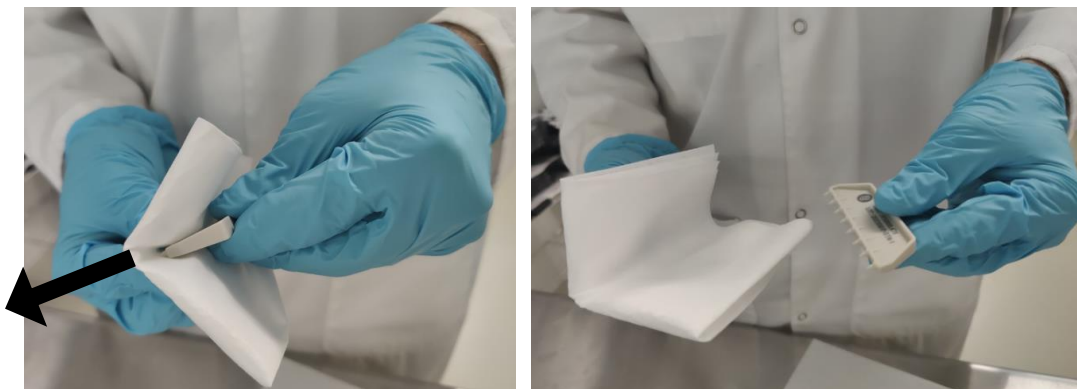


Figure 12 Cleaning the Piercing Tool

Between experiments, Stilla recommends storing the Piercing Tool in its provided transparent bag, to avoid dust deposits or RNA/DNA contamination.

The tool is ready for additional Crystal Digital PCR experiments.

Troubleshooting

1. Quantification from chambers with less than 10,000 droplets should be considered with caution and requires a careful observation of the Droplet Crystal to check the potential presence of artefacts affecting the results.
2. For any issues during the use of the Ruby Chip, please contact Technical Support.

Precautions and Warnings

Precautions

1. Wear protective clothing: nitrile or PVA (Polyethylene/Ethylene Vinyl Alcohol) gloves – safety glasses with side shield are recommended.
2. Avoid release of oil, or components contaminated with oil, into the environment.
3. Adsorb oil spills with a cleaning wipe or an inert liquid-binding material, dispose according to the precautions mentioned in the disposal considerations section.
4. Before the experiment, please be familiar with and master the operation methods and precautions of various instruments to be used and carry out quality control for each experiment.
5. Use general dilution ventilation and/or local exhaust ventilation.
6. Laboratory management should be strictly in accordance with the management regulations of PCR gene amplification laboratories.
7. All test samples are considered to be infectious. During the experiment, wear overalls, disposable gloves and change gloves frequently to avoid cross-contamination between samples. Sample handling and waste disposal must meet relevant regulatory requirements: General Guidelines on Biosafety of Microbial Biomedical Laboratories and Regulations on Medical Waste Management.

Warnings

1. In case of inhalation of emulsion oil: aerate with fresh air; if you feel unwell, seek medical help.
2. In case of skin contact with emulsion oil and surfactant: wash with soap and water. If signs/symptoms develop, seek medical help.
3. In case of eye contact with emulsion oil and surfactant: flush with large amounts of water. Remove contact lenses if easy to do. Continue rinsing. If signs/symptoms persist, seek medical help.
4. In case of emulsion oil and surfactant is swallowed: rinse the mouth. If you feel unwell, seek medical help.
5. In case of fire, use a fire-fighting agent, suitable for ordinary combustible materials such as water or foam, to extinguish.

Disposal Considerations



Must not be disposed with household garbage. Do not allow product to reach sewage system.

Dispose of waste product in a permitted industrial waste facility. Consult state local or national regulations for proper disposal.

Ruby Chip Limitations

- Strict compliance with this document is required for optimal results.
- Appropriate sample collection, transport, storage, and processing procedures are required for optimal results. Any improper handling and operation will cause inaccurate results.

Technical Support Contact Information

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

For help and technical advice or any issue regarding the Ruby Chip 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.

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Patents: www.stillatechnologies.com/patents/



Stilla Technologies
F-94800 Villejuif, FRANCE

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