### naica® PCR MIX

# Instructions for Use

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

#### Table 1. naica® PCR MIX ordering information

MIX	Buffer A	Buffer B	Number of reactions	Number of				
assembly	Concentration	Concentration	on Sapphire chips	reactions on Opal				
Reference				chips				
R10056	5X	100%	300	1050				
R10106	10X	100%	300	1050				

### **Resources**

The technical resources for the naica® PCR MIX are available at: <u>https://www.stillatechnologies.com/technical-resources/naica-system-prism6/</u>.

For more information and guidelines on setting up Crystal Digital PCR<sup>™</sup> experiments, please visit the company website <u>https://www.stillatechnologies.com/</u>.

### **Components**

The 5X and 10X naica® PCR MIX are each comprised of two components:

5X concentration:

Buffer A: 5X mix Buffer B: 100%

10X concentration: Buffer A: 10X mix







**Figure 1**. Components of the 10X naica<sup>®</sup> PCR MIX buffer tubes: A) Buffer A with green cap, and B) Buffer B with red cap.

### Storage

- Store Buffer A and Buffer B tubes at all times in an upright position. Stilla Technologies recommends placing the reagent tubes upon reception in an appropriate tube storage rack at the indicated storage temperatures.
- Store Buffer A at -20 °C +/- 5°C in original tubes. Do not aliquot in alternative tubes.
- Protect Buffer A from light. Buffer A can be thawed up to 20 times without observable deviations in performance.
- Store Buffer B at +20 °C +/- 5°C in original tubes. Do not aliquot in alternative tubes.
- Store Buffer B in a dark place protected from light.

Under these conditions, the naica® PCR MIX is stable for 12 months from the date of production. The respective expiration dates are specified on the naica® PCR MIX packaging and on the product labels.

General consideration for reagent storage: all tube caps should be well-closed before stocking.

# Description

The naica® PCR MIX has been developed for optimized performance for Crystal Digital PCR<sup>™</sup> on the naica® system. The naica® PCR MIX is compatible with Sapphire chips and Opal chips.

The naica® PCR MIX is a ready-to-use two-component solution (Buffer A and Buffer B) comprised of all the reagents necessary to perform fluorescent intercalating dye-based Crystal Digital PCR<sup>™</sup>, excluding DNA primers, the fluorescent DNA intercalating dye and DNA template.

The naica® PCR MIX is intended for use with the dye EvaGreen® in Crystal Digital PCR<sup>™</sup> with Sapphire chips and Opal chips using the naica® system.



The naica® PCR MIX 10X high concentration format reduces the total input volume of the mix, thus liberating a reaction volume that can be utilized by a high sample volume, leading to superior detection sensitivity of low concentration samples and rare targets.

The central component of the naica® PCR MIX is a highly efficient and processive hot start DNA polymerase. An initial denaturation step of 3 minutes at 95°C is sufficient to fully activate the polymerase. Stilla Technologies does not recommend using a longer initial denaturation step.

For Research Use Only. Not for use in diagnostic procedures. For use by professional personnel. Any additional use outside the described Instructions for Use of the naica® PCR MIX requires user validation.

### **Instrument Compatibility**

The naica® PCR MIX is intended for use with Stilla Technologies' Crystal Digital PCR<sup>™</sup> using the naica® system. The naica® PCR MIX is optimized for use with the fluorescent DNA intercalating dye EvaGreen® and is not suitable for use with dual labelled fluorescent probes. *Note:* SYBR® Green is not compatible with the naica® system.

### Guidelines for Crystal Digital PCR™

For the best efficiency in digital PCR, amplicons should ideally be no longer than 130 bp in length. Assay performance might be impaired with longer amplicons, particularly when using highly fragmented DNA templates (e.g., FFPE DNA or circulating DNA).

### **DNA Digestion**

DNA samples with  $\geq$ 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. DNA fragmentation by restriction digest is important in various applications and particularly important in copy number variation (CNV) analyses.

#### **Caution for FFPE Samples**

It is recommended to perform tests to select a compatible FFPE extraction protocol, as the extraction purity of the FFPE sample pre-processing may interfere with optimal performance of Crystal Digital PCR<sup>™</sup>.

### **Conditions for Use**

Buffer A should be operated at a temperature ranging from 4°C to 25°C.

Buffer B should be operated at a temperature ranging from 20°C to 25°C and protected from light.

All tube caps shall be well closed after use.



# **Reaction Protocol**

Buffer A: Before each use:



- thaw completely

- vortex thoroughly (suggested three times of 5-10 seconds each) and briefly centrifuge to collect the liquid at the bottom of the tube.

Buffer B: During assay optimization, it is recommended to start with a final concentration of 4% and not to exceed 5%. Typical final concentrations range from 2 to 5%.

Primers: before each use, thaw completely. Typical final concentrations range from 0.125 to 1 µM.

After combining all reagents, vortex thoroughly (suggested 5-10 seconds) to mix contents. Centrifuge briefly to collect the liquid at the bottom of the tube before loading the reaction mix in the consumable chips. Proceed directly to loading the reaction in the respective chip.

For Sapphire chips, the final well reaction volume is 25  $\mu$ L. For Opal chips, the final well reaction volume is 7  $\mu$ L.

# **Reaction Assembly**

Component	Final concentration	Volume					
		Sapphire chips		Opal chips			
		5X buffer A	10X buffer A	5X buffer A	10X buffer A		
Buffer A - naica® PCR MIX	1X	5 µL	2.5 µL	1.4 µL	0.7 µL		
Buffer B - naica® PCR MIX	4%*	1 µL		0.28 µL			
EvaGreen®, 20X	1.5X	1.9 µL		0.525 μL			
Dextran Alexa Fluor® 647, 0.02 mg/mL**	0.8 ng/µL	1 µL		0.28 µL			
Primers	Variable	Variable		Variable			
Template***	Variable	Up to 15.8 µL	Up to 18.3 µL	Up to 4.4 µL	Up to 5.1 µL		
Nuclease free water	NA	Complete to reach a final volume of 25 µL		Complete to reach a final volume of 7 µL			

\*Suggested final concentration, not to exceed 5%. Buffer B is provided at an initial concentration of 100%.

\*\* *In Crystal Digital PCR*<sup>™</sup>, a reference dye is used to increase the basal fluorescence of droplets and enable their detection by Crystal Reader or Crystal Miner software. When using EvaGreen®,



the basal fluorescence from the dye is usually sufficient to allow droplet detection in the blue channel. However, in case the red channel has to be used for droplet detection, the Dextran Alexa Fluor® 647 will serve as the reference dye.

\*\*\* Maximum template input volume is indicative and should be adapted to your actual input volumes of Buffer B, and primers.

### **Analysis**

For data acquisition and data analysis, Crystal Reader software and Crystal Miner software is required. The latest software versions, including the respective scanning templates and analysis configuration files corresponding to the naica® PCR MIX reagent, are available at the Technical Resources webpage.

### **Quality Control**

Each batch of naica® PCR MIX is tested according to EN ISO 13485:2016.

A Certificate of Compliance is available upon request from the Technical Support Department.

#### **Precautions and Warnings**

The naica® PCR MIX is not classified as dangerous according to Regulation (EC) No. 1272/2008 [CLP].

Appropriate personal protection equipment for handling this product, including lab coat, disposable gloves, and goggles, is required.

Wear additional personal protection equipment when needed. Wash hands before breaks and after work. Remove contaminated, saturated clothing.

In case of exposure:

General information: when in doubt or if symptoms are observed, get medical advice.

Following inhalation: no special measures are necessary. Provide fresh air.

Following skin contact: wash with soap and water.

Following eye contact: in case of eye irritation consult an ophthalmologist. Rinse immediately, carefully, and thoroughly with eyebath or water.

Following ingestion: if swallowed: rinse mouth. Do NOT induce vomiting.

Self-protection of the first aider : no special measures are necessary.

For further information, please refer to the material safety data sheet available on the technical resources webpage.

#### **Disposal Considerations**

Waste can be considered as a biohazardous waste and must be disposed of according to applicable domestic legislation.

For recycling, please contact the manufacturer.



## **Technical Support contact information**



Stilla Technologies F-94800 Villejuif, FRANCE For help and technical advice, please contact the Technical Support Department at Stilla Technologies.

For European Customers: Phone: (+33) 09 82 27 47 47 Email: <u>support@stilla.fr</u>

For North American Customers: Phone: 1-833-888-0150 ext.1 Email: <u>support@stilla-inc.com</u>

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

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