naica® PCR MIX

Instructions for Use

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

Product Name

naica® PCR MIX

Table 1. naica® PCR MIX ordering information

MIX assembly Reference	Buffer A Concentration	Buffer B Concentration	Number of reactions on Sapphire Chip	Number of reactions on Ruby Chip
R10056	5X	100%	300	1500
R10106	10X	100%	300	1500

Resources

Depending on the naica® system configuration, the technical resources for using the naica® PCR MIX are available:
3-color naica® system / Prism3 N10001.3
https://www.stillatechnologies.com/technical-resources/naica-system-prism3/
6-color naica® system / Prism6 N20001.6
https://www.stillatechnologies.com/technical-resources/naica-system-prism6/

Intended Use

The naica® PCR MIX is a component of the naica® system, a high-resolution genetic analysis system based on digital PCR. The naica® PCR MIX is intended for use by laboratory personnel trained to perform Crystal Digital PCR™ using the naica® system. For use by professional personnel. Any additional use outside the described Instructions for Use of the naica® PCR MIX requires user validation.

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Composition

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
- Buffer B: 100%

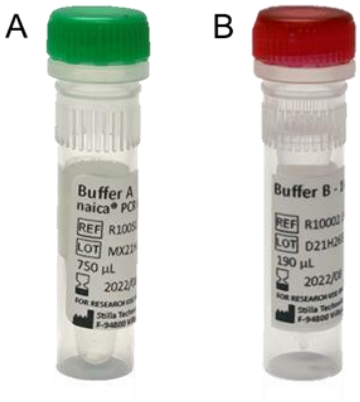

Figure 1. Components of the naica® PCR MIX buffer tubes: A) Buffer A with green cap, and B) Buffer B with red cap.

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™ on the naica® system. The naica® PCR MIX is compatible with Sapphire Chip and Ruby Chip.

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™, 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™ with Sapphire Chip and Ruby Chip 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.

The DNA polymerase used in the naica® PCR MIX is derived from bacteria and undergoes a standardized purification process to reduce contaminating bacterial DNA sequences from the enzyme preparation. However, bacterial DNA sequence contamination may be present in low quantities and should be verified on an application-specific basis.

Instrument Compatibility

The naica® PCR MIX is intended for use with Stilla Technologies' Crystal Digital PCR™ 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 ≥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: Input material for the Crystal Digital PCR™ workflow includes extracted nucleic acid. The purity of the extracted sample may vary depending on the raw material and the implemented extraction protocol. It is recommended to perform validation tests to select a compatible extraction protocol for optimal performance of Crystal Digital PCR™.

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.

Discard remaining components as soon as either Buffer is fully used. Do not use Buffers separately.

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. It is not recommended to freeze the combined reagent solution.

For Sapphire Chip, the final well reaction volume is 25 µL.

For Ruby Chip, the final well reaction volume is 5 µL.

Reaction Assembly

Component	Final Concentration	Volume			
		Sapphire Chip		*Ruby Chip*	
		5X Buffer A	*10X Buffer A*	*5X Buffer A*	*10X Buffer A*
Buffer A - naica® PCR MIX	1X	5 µL	2.5 µL	1 µL	0.5 µL
Buffer B - naica® PCR MIX	4%*	1 µL		0.2 µL	
EvaGreen®, 20X	1.5X	1.9 µL		0.375 µL	
Dextran Alexa Fluor® 647, 0.02 mg/mL**	0.8 ng/µL	1 µL		0.2 µL	
Primers	Variable	Variable		Variable	
Template***	Variable	Up to 15.8 µL	Up to 18.3 µL	Up to 3.1 µL	Up to 3.6 µL
Nuclease free water	NA	Complete to reach a final volume of 25 µL		Complete to reach a final volume of 5 µL	

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

** *In Crystal Digital PCR™*, 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 must 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.

When the blue channel is used for droplet detection, a scanning template named "ScanningTemplate_InstrumentName_ChipType_naica-PCR-MIX_**Evagreen**_version.ncx" must be selected prior scanning.

In case the red channel must be used for droplet detection, a scanning template named "ScanningTemplate_**Prism3**_ChipType_naica-PCR-MIX_**Evagreen**_**RED-detection**_version.ncx" or "ScanningTemplate_**Prism6**_ChipType_naica-PCR-MIX_**Evagreen**_**INFRA-RED-detection**_version.ncx" must be selected instead.

If after scanning chip(s) with the droplet recognition set in the blue channel, it appears that the reference channel must be switched from the blue one to the red one for a better droplet recognition, it is possible to directly reanalyze the experiment in Crystal Miner software without scanning the chip(s) again. Further details on experiment re-analysis are provided in the section "How to perform image re-analysis" of "Crystal Miner software" User Manual available in the Technical Resources of Stilla Technologies website.

Note: When using the red channel for droplet recognition, it is possible that droplets impacted by artefacts with a high level of fluorescence in the blue channel but invisible in the red channel, like dust particles, are not properly excluded from the analysis. This may lead to false positive dots in the dot-plots. It is thus necessary to screen for abnormally high fluorescent droplets in the dot-plots using the Explore Crystal feature of Crystal Miner software and manually exclude such droplets.

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

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

For help and technical advice or any issue regarding the naica® PCR MIX contact us:

For customers from Europe, China, and Africa:

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

Closed on French bank holidays.

Phone: (+33) 9 82 27 47 47

Email: support@stilla.fr.

For customers from America, Asia (without China) and Oceania

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

Closed on American bank holidays.

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

Email: support@stilla-inc.com

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Patents: www.stillatechnologies.com/patents/