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

Documents referenced in these Instructions for Use (IFU) are available here:

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

Intended Use

The naica® PCR MIX is a component of the naica® system and Nio™+, high-resolution genetic analysis systems based on digital PCR. The naica® PCR MIX is intended for use by qualified laboratory personnel trained to perform Crystal Digital PCR® using the naica® system or Nio™+. 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%

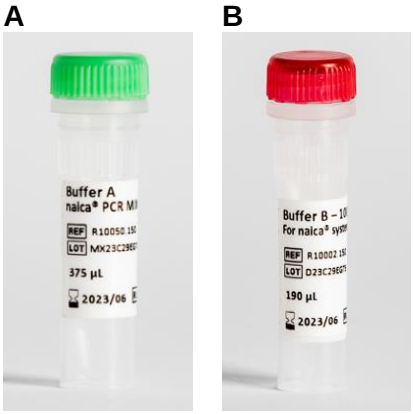

Figure 1. Components of the naica® PCR MIX buffer tubes: A) Buffer A with green cap, and B) Buffer B with red cap.

Storage

-  Store Buffer A and Buffer B tubes at all times in an upright position. It is recommended to store all tubes in the provided cardboard box or in an appropriate tube storage rack at the indicated storage temperatures.
- Store Buffer A and Buffer B at -20°C ± 5°C in original tubes. After opening, store Buffer B at +20°C ± 5°C in original tubes. Do not aliquot in alternative tubes.
- Buffer A can be thawed up to 20 times without observable deviations in performance.
-  Store Buffer A and Buffer B in a dark place protected from light**.**
- General consideration for reagent storage: all tube caps should be well-closed before stocking.

Under these conditions, the naica® PCR MIX is stable until the expiration date indicated on the external packaging label.

Description

The naica® PCR MIX has been developed for optimized performance for Crystal Digital PCR® on the naica® system and Nio™+. 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 and Nio™+.

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 and Nio™+. 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 and Nio™+.

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.
- Never combine Buffers from different naica® PCR MIX boxes.

Reaction Protocol

Buffer A: Before each use:

- Thaw completely
-  Vortex thoroughly (suggested three times of 5-10 seconds each at maximum speed) 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 and vortex thoroughly. Typical final concentrations range from 0.125 to 1 µM.

After combining all reagents, vortex thoroughly (suggested 10 seconds at maximum speed) 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, 20 µg/mL**	0.74 µg/mL	1 µL		N/A	
Dextran Alexa Fluor® 647, at 200 µg/mL**	7.4 µg/mL	N/A		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 the reading and analysis software. When using EvaGreen®, the basal fluorescence from the dye is usually sufficient to allow droplet detection in the Blue channel. However, in case basal fluorescence in Blue is not sufficient for accurate droplet detection, an alternative channel must be used. The Dextran Alexa Fluor® 647 will serve as the reference dye in the Red (Prism3) or Infra-Red (Prism6, Nio™+) channel.

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

Analysis

naica® system

For data acquisition and data analysis on the naica® system (Prism3 or Prism6), Crystal Reader software and Crystal Miner software are required. The latest software versions, including the respective scanning parameters and analysis configuration files corresponding to the naica® PCR MIX reagent, are available at the Technical Resources webpage.

When the basal fluorescence of the EvaGreen® dye in the Blue channel is used for droplet detection, select the following scanning template prior scanning:

“ScanningTemplate_InstrumentName_ChipType_naica-PCR-MIX_**Evagreen**_version.ncx”

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/Infra-Red one for a better droplet recognition, it is possible to directly re-analyze the experiment in Crystal Miner software without scanning the chip(s) again.

Dextran Alexa Fluor® 647 dye present in the PCR reaction mix provides a basal fluorescent level in all droplets in case a re-analysis is required for an accurate droplet detection in either Red with Prism3 or Infra-Red with Prism6. Further details on experiment re-analysis are provided in the section “How to perform image re-analysis” of the Crystal Miner User Manuals available in the Technical Resources of Stilla Technologies website.

For further experiments, when this alternative channel (Red for Prism3 or Infra-Red for Prism6) is used for droplet detection, one of the following scanning templates must be selected:

“ScanningTemplate_**Prism3**_ChipType_naica-PCR-MIX_**Evagreen_RED-detection**_version.ncx”

“ScanningTemplate_**Prism6**_ChipType_naica-PCR-MIX_**Evagreen_INFRA-RED-detection**_version.ncx”

Note: When using the Red/Infra-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/Infra-Red channel, like dust particles, are not properly excluded from the analysis. This may lead to false positive dots in the dot-plots. Thus, it is 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.

Nio™+

For data acquisition and data analysis on the Nio™+, Nio™ Reader and Nio™ Analyzer software are required. The latest software versions, including the respective scanning parameters and analysis configuration files corresponding to the naica® PCR MIX reagent, are available at the Technical Resources webpage.

Nio™+ protocol and Nio™+ assays to launch an Evagreen® run are directly available as templates in the Nio™ Reader software. When the basal fluorescence of the EvaGreen® dye in the Blue channel is used for droplet detection, select the following assay and protocol prior scanning:

- Protocol > New > Load an official Template: *Template_PCR-45-cycles_Evagreen-BlueDetection_naica-PCR-MIX_RubyChip_v0.nioprotocol*
- Assays > New > Load an official Template: *Template_naica-PCR-MIX_Evagreen_RubyChip_v0.nioassay*

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 Infra-Red one for a better droplet recognition, the chips must be re-scanned using:

- Protocol: same protocol as previous step but the **"Reading-only protocol"** check box must be activated by clicking on it in the Protocol page. The "Reading-only protocol" disables the PCR step and performs only a scan of the chips.
- Change the mix selection in the Protocol page under the panel Components > Mix type from **naica® PCR MIX (Blue reference)** to **naica® PCR MIX (Infra-Red reference)** to allow the detection of the droplets in Infra-Red instead of Blue.
- Assays > New > Load an official Template: *Template_naica-PCR-MIX_Evagreen_RubyChip_v0.nioassay*

Dextran Alexa Fluor® 647 dye present in the PCR reaction mix provides a basal fluorescent level in all droplets in case a scan with a droplet recognition in Infra-Red is required with Nio™+.

When this alternative channel is used for droplet detection, select for further experiments:

- Protocol > New > Load an official Template: *Template_PCR-45-cycles_Evagreen-InfraRedDetection_naica-PCR-MIX_RubyChip_v0.nioprotocol*
- Assays > New > Load an official Template: *Template_naica-PCR-MIX_Evagreen_RubyChip_v0.nioassay*

Note: the templates provided have by default 45 cycles of PCR with a hybridization temperature of 58°C. The PCR program should be adapted and validated according to the assay tested. The right channels for scanning are pre-selected in the templates. According to the assay tested, exposure times might need to be readjusted.

Note: When using the Infra-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 Infra-Red channel, like dust particles, are not properly excluded from the analysis. This may lead to false positive dots in the dot-

plots. Thus, it is necessary to screen for abnormally high fluorescent droplets in the dot-plots using the Explore Crystal feature of Nio™ Analyzer 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 an 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 national or local legislation. For recycling of cardboard packaging, please consult local or national regulations.

Technical Support Contact Information

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

For technical questions or any issue regarding the naica® PCR MIX, contact us:

Monday to Friday, 9:30 AM – 6:30 PM, Central European Time (CET).
Closed on French bank holidays.
Phone: (+33) 09 82 27 47 47
Email: support@stilla.fr

Patents: https://www.stillatechnologies.com/patents/

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Stilla Technologies
F-94800 Villejuif, FRANCE