naica® PCR MIX

Instructions for Use

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

Table 1. naica® PCR MIX ordering information

MIX assembly Reference	Buffer A Concentration	Buffer B Concentration	Number of reactions on Sapphire chips	Number of reactions on Opal chips
R10050	5X	100%	250	875
R10100	10X	100%	250	875

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
- Buffer B: 100%

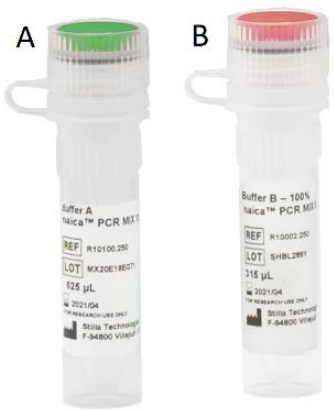

Figure 1. Components of the 10X naica® PCR MIX buffer tubes: A) Buffer A with green cap, and B) Buffer B with red cap.

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 10 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 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™, 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 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 requiring an activation time of 3 minutes at 95°C.

Please see this Technical Note for more details about using the naica® PCR MIX with the Sapphire chips.

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™ 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).

For more details and further guidelines on setting up Crystal Digital PCR™ experiments visit Stilla Technologies Online Learning Center – Gene-Pi https://www.gene-pi.com/items/ or the company website https://www.stillatechnologies.com/

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 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™.

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.

Please see the naica® system User Manuals for further concentration recommendations here: https://www.stillatechnologies.com/technical-resources/naica-system-prism3/.

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 µL.
For Opal chips, the final well reaction volume is 7 µL.

Visit https://www.gene-pi.com/item/primers-and-probes-2/ for more guidelines on PCR program design and optimization.

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*™, 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 (version 2.4.0.3), as well as running the "ScanningTemplatesUpdate_Prism3_202104" installation wizard are required. The installation wizard contains the latest versions of the respective Sapphire chip and Opal chip scanning templates and analysis configuration files corresponding to the naica® PCR MIX.
Detailed instructions for download of the required "ScanningTemplatesUpdate_Prism3_202104" installation wizard are available at https://www.stillatechnologies.com/technical-resources/naica-system-prism3/.

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 here:
https://www.stillatechnologies.com/naica-system-dpcr-mixes/.

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

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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: support@stilla.fr

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

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

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