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	Chip compatibility*
R10050	5X	100%	250	Sapphire
R10100	10X	100%	250	Sapphire

*the naica® PCR MIX is not validated on the Opal chip

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%

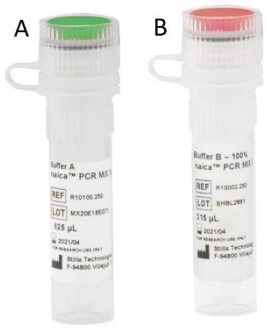

Figure 1. Components of the 10X naica® PCR MIX buffer tubes: A) Buffer A with green cap, and B) Buffer B with red cap.

Storage

Store Buffer A at -20 °C +/- 5°C in original tubes until the expiration date indicated on the label. Under these conditions, the naica® PCR MIX is stable for 12 months.

Buffer A can be thawed up to 10 times without observable deviations in performance.

Buffer B should be stored at +20°C +/- 5°C

All tube caps should be well closed before stocking.

Description

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™ on Sapphire chips, 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™ on Sapphire chips using the naica® system (read the Technical Note for more details). The naica® PCR MIX is available in a volume format corresponding to 250 reactions when used in conjunction with Sapphire chips, at both 5X

and 10X concentrations (Table 1). 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.

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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® (see naica® system User Manual https://www.stillatechnologies.com/technical-resources/ for details about Crystal Digital PCR™ using EvaGreen®) and is not suitable for use with dual labelled fluorescent probes. *Note: SYBR® Green is not compatible with the naica® system.*

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.

All tube caps shall be well closed after use.

Guidelines for Digital PCR

For details on setting up a Digital PCR experiment, visit https://www.gene-pi.com/items/ and https://www.stillatechnologies.com/
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).

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 0.25 to 0.5 µM, see naica® system User Manual https://www.stillatechnologies.com/technical-resources/ for further concentration recommendations.

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

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 chip.

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 (Sapphire chip)**
Buffer A - naica® PCR MIX	1X
Buffer B - naica® PCR MIX	4%*
Primers	Variable
EvaGreen®	1.5X to 3X
Dextran Alexa Fluor® 647**	0.8 ng/µL
Template	Variable
Nuclease free water	Up to 25 µL

*suggested final concentration, not to exceed 5%

** *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®, Dextran Alexa Fluor® 647 should always be added to the reaction mix.*

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.

Analysis

The analysis configuration yaml file (AnalysisConfiguration_naica_PCR_MIX_Prism3.yml) corresponding to the naica® PCR MIX must be selected via the Crystal Reader software and applied before scanning the chips to ensure proper image analysis and data quantification by Crystal Miner software. The analysis configuration file can be downloaded here: https://www.stillatechnologies.com/naica-system-dpcr-mixes/

Quality control

Free of detectable DNase.

Each batch of naica® PCR MIX is functionally tested in Crystal Digital PCR™ assays against a reference batch, and in qPCR using four serial dilutions of an internal positive control DNA.

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

Warning and Precautions

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 include lab coat, disposable gloves, and goggles (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.

Please refer to the safety data sheet for further information: https://www.stillatechnologies.com/wp-content/uploads/2020/09/SDS-EN-PCR-Mix-Naica-5X-10X-R10XXX_final.pdf

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: 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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