naica® multiplex PCR MIX

Instructions for Use

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

Product Name

naica® multiplex PCR MIX

Table 1. naica® multiplex 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
R10054	5X	100%	150	750
R10055	5X	100%	300	1500
R10104	10X	100%	150	750
R10105	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® multiplex PCR MIX is a component of the naica® system and Nio™+, high-resolution genetic analysis systems based on digital PCR. The naica® multiplex 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® multiplex PCR MIX requires user validation.

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Composition

The 5X and 10X naica® multiplex 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® multiplex PCR MIX buffer tubes: A) Buffer A with blue 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® multiplex PCR MIX is stable until the expiration date indicated on the external packaging label.

Description

The naica® multiplex PCR MIX has been developed for optimized performance for multiplexed Crystal Digital PCR® on the naica® system and Nio™+. The naica® multiplex PCR MIX is compatible with Sapphire Chip and Ruby Chip.

The naica® multiplex PCR MIX is a ready-to-use two-component solution (Buffer A and Buffer B) comprised of all the reagents necessary to perform fluorescent probe-based multiplex Crystal Digital PCR®, excluding DNA primers, DNA fluorescent probes, and DNA template.

The naica® multiplex PCR MIX is intended for use in fluorescently labelled probe-based multiplex Crystal Digital PCR® (such as TaqMan®) with Sapphire Chip and Ruby Chip using the naica® system and Nio™+.

The naica® multiplex PCR MIX 10X concentration format reduces the total input volume of the mix, thus liberating a reaction volume that can be utilized by the addition of multiple primers and probes and/or a high sample volume, leading to increased multiplexing capacity and superior detection sensitivity of low concentration samples and rare targets.

The central component of the naica® multiplex 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® multiplex 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® multiplex PCR MIX is intended for use with Stilla Technologies' Crystal Digital PCR® using the naica® system and Nio™+. The naica® multiplex PCR MIX contains an internal reference dye for partition detection and identification in the BLUE channel of the naica® system and Nio™+.

Guidelines for Crystal Digital PCR®

For the best efficiency in digital PCR using TaqMan® probes, 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 and protected from light.
- 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 all remaining components as soon as either Buffer is fully used.
- Never combine Buffers from different naica® multiplex 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 and Probes: 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 5-10 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® multiplex PCR MIX	1X	5 µL	2.5 µL	1 µL	0.5 µL
Buffer B - naica® multiplex PCR MIX	4%*	1 µL		0.2 µL	
Primers	Variable	Variable		Variable	
Probes	Variable	Variable		Variable	
Template**	Variable	Up to 18 µL	Up to 20.5 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%.

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

Analysis

For data acquisition and data analysis on the naica® system, Crystal Reader software and Crystal Miner software are required. For data acquisition and data analysis on the Nio™+, Nio™ Reader software and Nio™ Analyzer software are required.

The latest software versions, including the respective scanning parameters and analysis configuration files corresponding to the naica® multiplex PCR MIX, are available at the Technical Resources webpage.

Quality Control

Each batch of naica® multiplex 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® multiplex 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 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® multiplex PCR MIX, contact us:

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

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