



naica Multiplex ddPCR Mix Instructions for Use Version 1.0

Catalog Number	Product Reference Number	Product Description
12025254	R10055	naica 5x Multiplex ddPCR Mix, 2 x 0.75 mL
12025256	R10105	naica Multiplex 10x ddPCR Mix, 2 x 0.375 mL

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Introduction

The naica Multiplex Droplet Digital PCR (ddPCR™) Mix is optimized for use with the QX700 Droplet Digital PCR System, the Nio Digital PCR system, the naica system, and software applications. The mix is a ready-to-use two-component solution (Buffer A with the blue cap and Buffer B with the red cap) that is composed of all reagents necessary to perform fluorescent probe-based multiplex Droplet Digital PCR (ddPCR) (such as TaqMan) with Sapphire chip (naica system only) and RDG16 Cartridge consumables. The naica Multiplex ddPCR Mix is available in 5x and 10x concentrations.



Purpose and Intended Use

This document provides information and instructions for using the naica Multiplex PCR Mix.

Important: The QX700 ddPCR System and its associated components are intended for **research use only (RUO)**. Do **not** use them for diagnostic procedures. Laboratory personnel and other users must complete all required training before using the compatible systems.

About the naica Multiplex ddPCR Mix

The naica Multiplex ddPCR Mix is used with fluorescently labeled probe-based multiplex ddPCR with RDG16 Cartridges on a QX700 Droplet Digital PCR System, Nio Digital PCR system, and naica system.

Note: The mix can also be used with the Sapphire Chip on the naica system.

The central component of the naica Multiplex ddPCR Mix is a highly efficient and processive hot start DNA polymerase. An initial denaturing step of 3 min at 95° C is sufficient to fully activate the polymerase.











Bio-Rad does not recommend using a longer initial denaturing step.

Important: The DNA polymerase used in the mix is derived from bacteria and undergoes a standardized purification process to reduce contaminating bacterial DNA sequences when preparing the enzyme.

However, bacterial DNA sequence contamination might be present in low quantities and should be verified on an application-specific basis.

The 10x high concentration format reduces the total input volume of the mix, thus providing a reaction volume that can be utilized by the addition of multiple primers and probes and/or a high sample volume. This typically leads to increased multiplexing capacity and superior detection sensitivity for rare targets and in low concentration samples.

Symbols Lexicon

 <p>Manufacturer</p>	 <p>Distributor</p>
 <p>Catalog Number</p>	 <p>Batch Code</p>
 <p>Use By Date</p>	 <p>Temperature Limit</p>
 <p>Do Not Use if Package is Damaged and Consult Instructions for Use</p>	 <p>Keep Away from Sunlight</p>
 <p>Consult Instructions for Use</p>	 <p>This Way Up</p>

Safety Requirements

For professional use in a laboratory environment only.

Danger: the naica Multiplex ddPCR Mix is classified as hazardous according to Regulation (EC) No. 1272/2008 [CLP]:

Contains Ovalbumins; 3(2H)-Isothiazolone, 2-methyl-



Hazard Statements

H317: May cause an allergic skin reaction

H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled

Precautionary Statements

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352: IF ON SKIN: Wash with soap and water.

P342+P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.

P501: Dispose of contents/container in accordance with local, regional, national and international regulations.

Handle with Good Laboratory Practices (GLP).

- Do not eat, drink or smoke when using this product.
- Always wear appropriate personal protection equipment for handling this product - lab coat, disposable gloves, and appropriate eye/face protection are required. Whenever needed, wear additional personal protection equipment.
- Contaminated work clothing should be removed immediately and not be allowed out of the workplace until decontaminated. Change disposable gloves frequently and to prevent cross-contamination.
- Before breaks and after work, always wash your hands.
- All materials of human origin are to be treated as potentially infectious. Handle samples based on Standard and Universal Precautions, following local, regional, and national guidelines (such as Biosafety in Microbiological and Biomedical Laboratories). Dispose of all samples according to biohazardous and medical waste management regulations.
- Handle with general biosafety laboratory ventilation.

In case of exposure:

- General information: Call a doctor/physician or POISON CENTER if you feel unwell.
- In case of skin contact, wash with soap and water. If skin irritation or a rash occurs, seek medical advice/attention.
- In case of eye contact, rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. If symptoms persist, consult an ophthalmologist.

- If inhaled, provide fresh air.
- If swallowed, rinse the mouth.
- Self-protection of the first aider: no special measures are necessary.
- In case of fire, use water, foam, or another agent suitable for ordinary combustibles.
- Clean up all spills immediately and thoroughly. Decontaminate the area for any spills involving biohazardous materials.

Refer to the Safety Data Sheets (SDS) for more safety information. The Safety Data Sheets are available at bio-rad.com and on request (contact Bio-Rad Technical Support).

Quality Control

Each batch of naica Multiplex ddPCR Mix is tested according to EN ISO 13485:2016. For a copy of the Certificate of Compliance, contact Bio-Rad Technical Support.

Disposal Requirements

Dispose of all kit components and contaminated materials appropriately and per all pertinent regulations.

Waste classified as biohazardous must be disposed of in compliance with relevant laboratory, local and or national requirements / regulations.

To recycle cardboard packaging, follow the requirements applicable to your laboratory or location.

Mix Requirements and Compatibility

Instrument Compatibility

The naica Multiplex ddPCR Mix is intended for use with the QX700 Droplet Digital PCR System, the Nio Digital PCR system, and the naica system. The naica Multiplex ddPCR Mix contains an internal reference dye for partition detection and identification in the Blue channel of these systems.

Usage Requirements

- Do not combine buffers from different naica Multiplex ddPCR Mix boxes.
- The buffers require the following operating temperature ranges: Buffer A: From 4°C to 25°C. Do not aliquot into other tubes.
- Buffer B: From 20°C to 25°C
- Ensure that Buffer A and Buffer B are protected from light.
- Ensure that all tube caps are securely closed after use.
- As soon as either buffer is fully used, discard the remaining components.

Storage Requirements

You must comply with the following requirements to ensure the naica Multiplex ddPCR Mix is stable until the expiration date shown on the external packaging label.

- *Without exception*, store the buffer tubes in an upright position.

Note: Bio-Rad recommends that you keep the original cardboard box, or provide an appropriate tube storage rack, to store the tubes at the indicated storage temperatures.

- Store Buffer A and Buffer B at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in their original tubes.
- You can thaw Buffer A up to 20 times without observable deviations in performance.
- After opening, store Buffer B at $+20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in its original tubes. Do not aliquot in alternative tubes.
- Store Buffer A and Buffer B in a dark place that is protected from light.
- Ensure that all tube caps are securely closed before storing.

ddPCR Guidelines

To maximize efficiency when using TaqMan probes, amplicons should (ideally) not exceed a length of 130 bp.

Assay performance might be impaired with longer amplicons, particularly when using highly fragmented DNA templates (for example, FFPE DNA or circulating DNA).

DNA Digestion Restriction

DNA fragmentation by digestion restriction is important in various applications and particularly important in copy number variation (CNV) analyses.

To ensure even distribution of the DNA template during partitioning, DNA samples with ≥ 10 kb average length (for example, genomic DNA) should be fragmented by restricting digestion before partitioning. Digestion restriction is not required for highly fragmented DNA (for example, FFPE DNA or circulating DNA), but you must use restriction enzymes that do not cut within the amplified sequence.

Important: Input material for the ddPCR workflow includes extracted nucleic acid. The purity of the extracted sample can vary, depending on the raw material and the implemented extraction protocol. For optimal ddPCR performance, Bio-Rad recommends performing validation tests to select a compatible extraction protocol.

Reaction Protocol Best Practices

- Before each use, thaw Buffer A completely and vortex thoroughly (suggested 3x, 5-10 sec each, at maximum speed), and then briefly centrifuge to collect the liquid at the bottom of the tube.
- Begin with a final concentration of 4% of Buffer B and do not exceed 5% during assay optimization. Typical final concentrations range from 2–5%.
- Before each use, thaw primers and probes completely and vortex thoroughly. Typical final concentrations range from 0.125 to 1 μM
- After combining all reagents, vortex thoroughly (suggested 5 to 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 cartridge. Bio-Rad recommends not to freeze the combined reagent solution.
- For the Sapphire Chip, the final well reaction volume is 25 μL .
- For RDG16 Cartridges, the final well reaction volume is 5 μL .

Reaction Assembly

Component	Final Concentration	Volume			
		Sapphire Chip		RDG16 Cartridge	
		5x Buffer A	10x Buffer A	5x Buffer A	10x Buffer A
Buffer A, naica Multiplex ddPCR Mix	1X	5 µL	2.5 µL	1 µL	0.5 µL
Buffer B, naica Multiplex ddPCR Mix	4% ⁽¹⁾	1 µL	1 µL	0.2 µL	0.2 µL
Primers and Probes	Variable	Variable	Variable	Variable	Variable
Template ⁽²⁾	Variable	Up to 18 µL	Up to 20.5 µL	Up to 3.1 µL	Up to 3.6 µL
Nuclease-free water	N/A	Complete to final volume of 25 µL		Complete to final volume of 5 µL	

⁽¹⁾ Suggested final concentration, not to exceed 5%. Buffer B is provided at an initial concentration of 100%.

⁽²⁾ 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 on the QX700 ddPCR System, the QX700 ddPCR System Control Software, and QX700 ddPCR System Analysis Software are required. For data acquisition and data analysis on the Nio Digital PCR system, the Nio Reader software and Nio Analyzer software are required. For data acquisition and data analysis on the naica system, the Crystal Reader software and Crystal Miner software are required.

For the latest software versions, which include respective scanning parameters and analysis configuration files corresponding to the naica Multiplex ddPCR Mix reagent, contact Bio-Rad Technical Support.

Documentation

- QX700 Droplet Digital PCR System Instrument Guide (DIR No. 10000171493)
- QX700 ddPCR System Analysis Software User Guide (DIR No. 10000171494)

Contacting Technical Support

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

Phone: 1-800-424-6723, option 2

E-mail: Support@bio-rad.com (U.S./Canada Only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at www.bio-rad.com.

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