



naica ddPCR Mix Instructions for Use Version 1.0

Catalog Number	Product Reference Number	Product Description
12025257	R10056	naica 5x ddPCR Mix, 2 x 0.75 mL
12025258	R10106	naica 10x ddPCR Mix, 2 x 0.375 mL

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Introduction











The naica Droplet Digital PCR (ddPCR™) Mix is optimized for use with the QX700 ddPCR System and software applications. The mix is a ready-to-use two-component solution containing Buffer A (with the green cap) and Buffer B (with the red cap), which are composed of necessary master mix reagents for performing ddPCR with fluorescent DNA intercalating dye EvaGreen®.

Additionally, the naica ddPCR Mix is compatible with:

- EvaGreen® dye with the Sapphire Chip and the RDG16 Cartridge on the naica system
- EvaGreen® dye with the RDG16 Cartridge on the Nio Digital PCR system
- with Crystal Universal Reporters for Crystal Flex Probes detection using the RDG16 Cartridge on the Nio Digital PCR system and on the naica system

The naica ddPCR Mix is available in 5x and 10x concentrations.

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 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 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 ddPCR Mix is compatible with:

- EvaGreen® dye with the RDG16 Cartridge on the QX700 ddPCR System
- EvaGreen® dye with the Sapphire Chip and the RDG16 Cartridge on the naica system
- EvaGreen® dye with the RDG16 Cartridge on the Nio Digital PCR system
- Crystal Universal Reporters for Crystal Flex Probes detection using the RDG16 Cartridge on the Nio Digital PCR system and on the naica system

Important: The naica ddPCR Mix is optimized for use with the EvaGreen® fluorescent DNA intercalating dye and is not suitable for use with **dual-labeled** fluorescent probes. *SYBR® Green is **not** compatible with the naica or QX700 Droplet Digital PCR systems.*

For detection using TaqMan probes, you can use naica Multiplex ddPCR Mix with the QX700 ddPCR System, Nio Digital PCR system, and the naica system.

Usage Requirements

- Do **not** combine buffers from different naica ddPCR Mix boxes.
- The buffers require the following operating temperature ranges:
 - Buffer A: From 4°C to 25°C
 - Buffer B: From 20°C to 25°C
- Ensure that Buffer B is 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 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. Do not aliquot into other tubes.
- After opening, store Buffer B at $+20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in its original tubes. Do not aliquot into other 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, 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

For detection with EvaGreen® fluorescent DNA intercalating dye, follow the instructions in this section. For detection with Crystal Flex Probes, refer to the ddPCR assay instructions for use and the respective Assay Information Sheet.

Before you begin, note the following:

- 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 completely and vortex thoroughly. Typical final concentrations range from 0.125 to 1 μL .
- After combining all reagents, vortex thoroughly (suggested 10 sec 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 load the reaction in the respective cartridge. It is not recommended to freeze the combined reagent solution.
- For 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 ddPCR Mix	1X	5 µL	2.5 µL	1 µL	0.5 µL
Buffer B, naica ddPCR Mix	4% ⁽¹⁾	1 µL	1 µL	0.2 µL	0.2 µL
EvaGreen®, 20X	1.5x	1.9 µL	1.9 µL	0.375 µL	0.375 µL
Dextran Alexa Fluor 647 20 µg/mL	0.74 µg/mL	1 µL	µL	N/A	N/A
Dextran Alexa Fluor 647 200 µg/mL ⁽²⁾	7.4 µg/mL	N/A	N/A	0.2 µL	0.2 µL
Primers	Variable	Variable	Variable	Variable	Variable
Template ⁽³⁾	Variable	Up to 15.8 µL	Up to 18.3 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⁾ In ddPCR, 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 FAM (Blue) channel. However, in case basal fluorescence in FAM (Blue) is not sufficient for accurate droplet detection, an alternative channel must be used. The Dextran Alexa Fluor® dye serves as the reference dye in the Red (Prism3) channel and Dextran Alexa Fluor® as the reference dye in the Infra-Red (Prism6, QX700) channel.

⁽³⁾ Maximum template input volume is indicative and should be adapted to your actual input volumes of Buffer B, and primers.

Analysis

For detection with fluorescent DNA intercalating dye (EvaGreen®), follow the instructions in this section. For detection with Crystal Flex probes, refer to the ddPCR assay instructions for use and the respective Assay Information Sheet.

Analysis Using the QX700 Droplet Digital PCR System

For data acquisition and data analysis on the QX700 Droplet Digital PCR System, QX700 ddPCR System Control Software, and QX700 ddPCR System Analysis Software are required. For the latest software versions, scanning parameters, and analysis configuration files, contact Bio-Rad Technical Support.

To launch an EvaGreen® run, you can use .nioprotocol and .nioassay files as templates in the QX700 ddPCR System Control Software. When the basal fluorescence of the EvaGreen® dye in the FAM (Blue) channel is used for droplet detection, select the protocol and assay specified in Steps 1 and 2 before scanning.

1. Select Protocol > New > Load an official Template, and then select the following:
Template_PCR-45-cycles_Evagreen-BlueDetection_naica-PCR-MIX_RubyChip.nioprotocol
2. Select Assays > New > Load an official Template, and then select the following:
Template_naica-PCR-MIX_Evagreen_RubyChip.nioassay

Important: If, after scanning cartridges with the droplet recognition set in the FAM (Blue) channel, it appears that you must switch to Dextran Alexa Fluor 647 (Infra-Red) for better droplet recognition, the chips must be re-scanned using the same protocol as the previous step, but with the following changes:

- a. Select the Reading-only protocol checkbox to disable the PCR step and perform only a scan of the chips.
- b. To allow droplet detection in Dextran Alexa Fluor 647 (Infra-Red) select Components > Mix type and then change the selected mix from naica ddPCR Mix (FAM-Blue reference) to naica ddPCR Mix (Dextran Alexa Fluor 647 -InfraRed reference).

When Dextran Alexa Fluor 647 dye is present in the PCR reaction mix, it provides a basal fluorescent level in all droplets in case a scan with a droplet recognition in Dextran Alexa Fluor 647 (Infra-Red) is required. When the alternative channel is used for droplet detection, complete the following steps for additional experiments:

1. Select Protocol > New > Load an official Template, and then select the following:
Template_PCR-45-cycles_Evagreen-InfraRedDetection_naica-PCR-MIX_RubyChip.nioprotocol
2. Select Assays > New > Load an official Template, and then select the following:
Template_naica-PCR-MIX_Evagreen_RubyChip.nioassay

Note the following:

- By default, the templates provided are configured with 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 correct channels for scanning are pre-selected in the templates.
- According to the assay tested, exposure times might need to be readjusted.

When using the Dextran Alexa Fluor® (Red)/ Dextran Alexa Fluor® (Infra-Red) channel for droplet recognition, droplets that display a high level of fluorescence in the Blue channel (but are invisible in the Infra-Red channels (like dust particles), are not properly excluded from the analysis.

Because this can lead to false-positive dots in the dot plots, you must screen for abnormally high fluorescent droplets in the dot-plots using the Explore Crystal feature in the QX700 ddPCR System Analysis Software, and then manually exclude the droplets.

Analysis Using the Nio Digital PCR System

For data acquisition and data analysis on the Nio Digital PCR system, Nio Reader and Nio Analyzer Software are required. For the latest software versions, scanning parameters, and analysis configuration files, contact Bio-Rad Technical Support.

To launch an EvaGreen® run, you can use .nioprotocol and .nioassay files as templates in the Nio Reader Software. When the basal fluorescence of the EvaGreen® dye in the FAM (Blue) channel is used for droplet detection, select the protocol and assay specified in Steps 1 and 2 before scanning.

1. Select Protocol > New > Load an official Template, and then select the following:
Template_PCR-45-cycles_Evagreen-BlueDetection_naica-PCR-MIX_RubyChip.nioprotocol
2. Select Assays > New > Load an official Template, and then select the following:
Template_naica-PCR-MIX_Evagreen_RubyChip.nioassay

Important: If, after scanning cartridges with the droplet recognition set on the FAM (Blue) channel, it appears that you must switch to Dextran Alexa Fluor 647 (Infra-Red) for better droplet recognition, the chips must be re-scanned using the same protocol as the previous step, but with the following changes:

- Select the Reading-only protocol checkbox to disable the PCR step and perform only a scan of the chips.
- To allow droplet detection in Dextran Alexa Fluor 647 (Infra-Red), select Components > Mix type and then change the selected mix from naica ddPCR Mix (Blue reference) to naica ddPCR Mix (InfraRed reference).

When Dextran Alexa Fluor 647 (Infra-Red) dye is present in the PCR reaction mix, it provides a basal fluorescent level in all droplets in case a scan with a droplet recognition in Dextran Alexa Fluor 647 (Infra-Red) is required. When the alternative channel is used for droplet detection, complete the following steps for additional experiments:

1. Select Protocol > New > Load an official Template, and then select the following:
Template_PCR-45-cycles_Evagreen-InfraRedDetection_naica-PCR-MIX_RubyChip.nioprotocol
2. Select Assays > New > Load an official Template, and then select the following:
Template_naica-PCR-MIX_Evagreen_RubyChip.nioassay

Note the following:

- By default, the templates provided are configured with 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 correct channels for scanning are pre-selected in the templates.
- According to the assay tested, exposure times might need to be readjusted.

When using the Dextran Alexa Fluor 647 (Infra-Red) channel for droplet recognition, droplets that display

a high level of fluorescence on the FAM (Blue) channel (but are invisible in the Dextran Alexa Fluor 647 (Infra-Red) channels (like dust particles), are not properly excluded from the analysis.

Because this can lead to false-positive dots in the dot plots, you must screen for abnormally high fluorescent droplets in the dot-plots using the Explore Crystal feature in the Nio Analyzer Software, and then manually exclude the droplets.

Analysis Using the naica system

For data acquisition and data analysis on the naica system (Prism3 or Prism6), Crystal Reader software and Crystal Miner software are required. For the latest software versions, including the respective scanning parameters and analysis configuration files corresponding to the naica ddPCR Mix, contact Bio-Rad Technical Support.

EvaGreen

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

ScanningTemplate_InstrumentName_ChipType_naica-PCR-MIX_Evagreen_version.ncx

If, after scanning cartridges with the droplet recognition set in the FAM (Blue) channel, it appears that you must switch to Dextran Alexa Fluor 647 (Infra-Red) for better droplet recognition, you can reanalyze the experiment in Crystal Miner Software without rescanning the cartridges.

When Dextran Alexa Fluor 647 (Infra-Red) dye is present in the PCR reaction mix, a basal fluorescent level is provided in all droplets in case a reanalysis is required for an accurate droplet detection in either Dextran Alexa Fluor 647 (Red) with Prism3 or Dextran Alexa Fluor 647 (Infra-Red) with Prism6.

Further details on experiment reanalysis are provided in the section "How to perform image re-analysis" of the Crystal Miner User Manuals. For more information, contact Bio-Rad Technical Support.

Dextran Alexa Fluor 647

When the Dextran Alexa Fluor 647 for Prism3 or Dextran Alexa Fluor 647 for Prism6 alternative channel is used for droplet detection, you must select one of the following scanning templates:

- ScanningTemplate_Prism3_ChipType_naica-PCR-MIX_Evagreen_RED-detection_version.ncx
- ScanningTemplate_Prism6_ChipType_naica-PCR-MIX_Evagreen_INFRA-RED-detection_version.ncx

When using the Dextran Alexa Fluor 647 (Infra-Red) channel for droplet recognition, droplets that display a high level of fluorescence in the FAM (Blue) channel (but are invisible in the Dextran Alexa Fluor 647 (Infra-Red) channels (like dust particles), are not properly excluded from the analysis.

Because this can lead to false-positive dots in the dot plots, you must screen for abnormally high fluorescent droplets in the dot-plots using the Explore Crystal feature in the Crystal Miner Software, and then manually exclude the droplets.

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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