

ddPCR™ Copy Number Variation Assays

Catalog # Description

10042958 ddPCR CNV Assay (FAM), 200 x 20 µL reactions
10042959 ddPCR CNV Assay (FAM), 1,000 x 20 µL reactions
10042960 ddPCR CNV Assay (FAM), 2,500 x 20 µL reactions
10042961 ddPCR CNV Assay (HEX), 200 x 20 µL reactions
10042962 ddPCR CNV Assay (HEX), 1,000 x 20 µL reactions
10042963 ddPCR CNV Assay (HEX), 2,500 x 20 µL reactions
12005520 ddPCR CNV Assay (Cy5), 200 x 20 µL reactions
12005571 ddPCR CNV Assay (Cy5), 1,000 x 20 µL reactions
12005572 ddPCR CNV Assay (Cy5), 2,500 x 20 µL reactions
12005573 ddPCR CNV Assay (Cy5.5), 200 x 20 µL reactions
12005574 ddPCR CNV Assay (Cy5.5), 1,000 x 20 µL reactions
12005575 ddPCR CNV Assay (Cy5.5), 2,500 x 20 µL reactions
12017373 ddPCR CNV Assay (ROX), 200 x 20 µL reactions
12017431 ddPCR CNV Assay (ROX), 1,000 x 20 µL reactions
12017424 ddPCR CNV Assay (ROX), 2,500 x 20 µL reactions
12017423 ddPCR CNV Assay (ATTO 590), 200 x 20 µL reactions
12017422 ddPCR CNV Assay (ATTO 590), 1,000 x 20 µL reactions
12017420 ddPCR CNV Assay (ATTO 590), 2,500 x 20 µL reactions
12025604 ddPCR CNV Assay (ATTO 550), 200 x 20 µL reactions
12025633 ddPCR CNV Assay (ATTO 550), 1,000 x 20 µL reactions
12025588 ddPCR CNV Assay (ATTO 550), 2,500 x 20 µL reactions

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Description

ddPCR Copy Number Variation (CNV) Assays are expertly designed specifically for Droplet Digital™ PCR (ddPCR) using proprietary computational algorithms. These assays have not been wet-lab validated by Bio-Rad Laboratories, Inc. Performance of these assays should be validated prior to use. Target and reference assays can be ordered with FAM, HEX, Cy5, Cy5.5, ROX, ATTO 550, and ATTO 590 fluorophores.

Ordering Information

Visit [bio-rad.com/digital-assays](https://www.bio-rad.com/digital-assays) to order ddPCR CNV Assays.

Storage and Stability

ddPCR CNV Assays are stable for 12 months when stored at 4°C protected from light. The 20x assay mix can be kept at -20°C for long-term storage.

Kit Contents

The ddPCR CNV Assay is a 20x concentrated, ready-to-use primer-probe mix. Each kit comes with 200, 1,000, or 2,500 µL of the 20x assay mix (18 µM primers and 5 µM probe), sufficient for 200, 1,000, or 2,500 x 20 µL reactions, respectively. For assays performed on the QX700™ ddPCR System, this would correspond to four times the number of reactions listed, as based on a final sample input volume of 5 µL in RDG16 Cartridges.

Reagents and Equipment

For assays using the QX200™ Droplet Generator (catalog #1864002) or Automated Droplet Generator (#1864101):

- For 1–2 targets, ddPCR Supermix for Probes (No dUTP) (#1863023, #1863024, #1863025) is recommended
- For >2 targets, ddPCR Multiplex Supermix (#12005909, #12005910, #12005911) is recommended
- QX200 Droplet Reader (#1864003) or QX600™ Droplet Reader (#12013328)
- PX1 PCR Plate Sealer (#1814000)

For assays using the QX ONE™ Droplet Digital PCR System (#12006536):

- ddPCR Multiplex Supermix (#12005909, #12005910, #12005911)
- PX1 PCR Plate Sealer (#1814000)

For assays using the QX Continuum™ Droplet Digital PCR System (#12019613):

- QX Continuum™ ddPCR Supermix for Probes (#12019028, #12019141, #12019133)
- PX1 PCR Plate Sealer (#1814000)

For assays using QX700 Droplet Digital PCR Systems (#17011036, #17010638, #17010628):

- naica™ 5X Multiplex ddPCR Mix (#12025253, #12025254)
- naica 10X Multiplex ddPCR Mix (#12025255, #12025256, #12025258)
- PX1 PCR Plate Sealer (#1814000)

Visit [bio-rad.com/ddPCRconsumables](https://www.bio-rad.com/ddPCRconsumables) to find ordering information on consumables such as oils, cartridges, gaskets, plates, and seals. Refer to Table 1 for fluorophore compatibility with instruments.



Table 1. Fluorophore compatibility.

QX200 Droplet Reader	QX ONE ddPCR System	QX Continuum ddPCR System	QX600 Droplet Reader	QX700 ddPCR System
FAM	FAM	FAM	FAM	FAM
HEX	HEX	HEX	HEX	HEX
	Cy5	ROX	ROX	ATTO 550
	Cy5.5	Cy5	ATTO 590	ROX
			Cy5	ATTO 590
			Cy5.5	Cy5
				Cy5.5

Determination of Optimal Annealing Temperature

Newly designed ddPCR CNV Assays should be run across a thermal gradient (55–65°C) to determine the annealing/extension temperature that optimizes separation between positive and negative droplets while minimizing rain (droplets that fall between the major positive and negative populations). If possible, an annealing/extension temperature that optimizes the performance of both target and reference assays should be selected.

- Using a test sample as template, prepare reaction mix for at least 8 wells (1 column) according to the guidelines in the Reaction Setup section
- For optimal performance, follow the recommendations in the Restriction Enzyme Digestion of Sample DNA and Reaction Setup sections
- After droplet generation, proceed to thermal cycling on a C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module (#1851197). Use an annealing temperature gradient as described in Table 2 for QX200, QX ONE, or QX600 Systems. See Table 3 for recommendations for the QX Continuum System and Table 4 for the QX700 System
- The optimal annealing temperature range is determined based on the separation between 4 clusters (Figure 1)
- For more information, see the Copy Number Variation Analysis section in the Droplet Digital PCR Applications Guide (bulletin 6407)

Table 2. Thermal gradient cycling conditions. Conditions to determine optimal annealing/extension temperatures on the QX200, QX ONE, or QX600 Droplet Digital PCR System.*

Cycling step	Temperature, °C**	Time	Number of cycles	
Hold (QX ONE ddPCR System only)	25	3 min	1	
Enzyme activation	95	10 min	1	
Denaturation	94	30 sec	40	
Annealing/extension	55–65	1 min	40	
Enzyme deactivation	98	10 min	1	
Hold	QX200 or QX600 ddPCR System (optional)	4	Infinite	1
	QX ONE ddPCR System (required)	25	1 min	1

* For the C1000 Touch Thermal Cycler, use a heated lid set to 105°C and set the sample volume to 40 µL.

** Check/adjust ramp rate settings to ~2°C/sec.

Table 3. Thermal cycling conditions for the QX Continuum ddPCR System.

Thermal cycling zone	Recommended temperature (°C)
Reverse transcription	50
Activation	95
Denaturation	95
Annealing	60
Extension	60

Table 4. Thermal cycling conditions for the QX700 ddPCR System.

Steps	Temperature (°C)*	Time
Step 1	95	180 sec
Step 2	Begin loop for 45 iterations	
Step 3	95	10 sec
Step 4	60**	15 sec

*Use a ramp rate of 1°C/sec for each step.

**Temperature may need to be adjusted to maximize separation between positive and negative droplets.

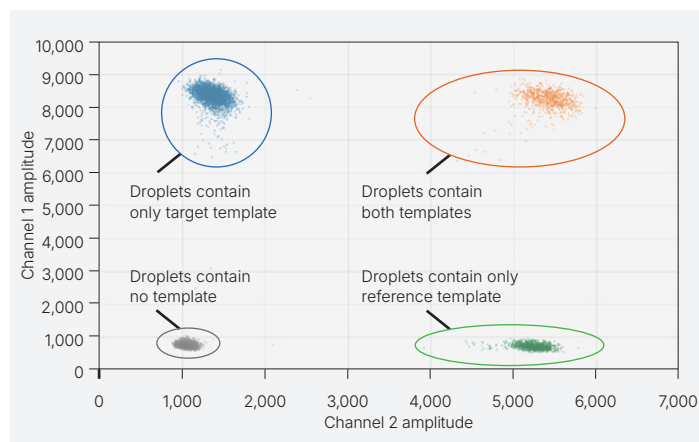


Fig. 1. Two-dimensional scatter plot for a QX200 ddPCR System. The scatter plot shows the four clusters obtained with a target (FAM) and reference allele (HEX).

Restriction Enzyme Digestion of Sample DNA

- DNA fragmentation by restriction digestion prior to droplet generation enables optimal accuracy by separating tandem gene copies, reducing sample viscosity, and improving template accessibility. Two strategies may be used to perform restriction digestion of DNA samples: digestion directly in the ddPCR reaction during setup (recommended) or conventional digestion prior to Droplet Digital PCR

Digestion Directly in Droplet Digital PCR

- Efficient digestion of sample DNA can be achieved by direct addition of restriction enzyme to the reaction
- 2–5 units of restriction enzyme per reaction is recommended
- Dilute the restriction enzyme using the recommended diluent buffer according to the manufacturer's instructions, and then add 1 µL to the reaction according to the guidelines in Table 3
- Reactions can be set up at room temperature; no additional incubation time is required

- The addition of restriction enzyme buffers with high salt content can inhibit Droplet Digital PCR and should be avoided

Digestion Prior to Droplet Digital PCR

- Restriction enzyme digestion can be carried out as a separate reaction before reaction setup
- Use 10–20 units of restriction enzyme per microgram of genomic DNA (gDNA)
- Incubate the reaction for 1 hr at the temperature recommended for the restriction enzyme
- Heat inactivation is not required but can be considered if long-term storage is required; do not heat inactivate above 65°C
- DNA purification is not necessary after restriction digestion
- Use a minimum 10-fold dilution of the digest to reduce the salt content of the sample in Droplet Digital PCR
- Store digested DNA at –20°C or below

Reaction Setup

1. For most routine CNV applications where a diploid target copy number is expected to be 10 or less, ≤50 ng of human gDNA should be added per ddPCR well. If 10–50 copies per diploid genome are expected in a sample, add ≤15 ng of sample per well. For copy number evaluation >50 copies per diploid genome, strategies using multiple wells can be used.
2. Thaw all components to room temperature. Mix thoroughly by vortexing each tube to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of each tube and store protected from light.
3. Prepare samples at the desired concentration before setting up the reaction mix according to the guidelines in Table 5. An example reaction setup for the QX200, QX600, or QX ONE ddPCR System is provided in Table 5. For instructions on reaction setup for the QX Continuum ddPCR System, refer to Table 6. For the QX700 System, see Table 7. If multiple samples are to be assayed using the same target and reference duplex, prepare a master reaction mix without the sample template, dispense equal aliquots into reaction tubes, and add the sample template to each reaction tube as the final step.
4. Mix thoroughly by vortexing each tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes. Allow the reaction tubes to equilibrate at room temperature for about 3 min.
5. Transfer the reaction mix from the reaction tubes to the appropriate ddPCR cartridge as follows:
 - a. For the QX200 Droplet Generator, load 20 µL of each reaction mix into the sample wells of DG8 Cartridges. Follow subsequent instructions as specified in the QX200 Droplet Generator Instruction Manual (10031907)
 - b. For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (10043138)

- c. For the QX ONE ddPCR and QX Continuum ddPCR Systems, load 20 µL of each reaction mix into the sample wells of GCR96 Cartridges. Follow subsequent instructions as specified in the QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide (10000116512)
- d. For the QX Continuum ddPCR System, load 16 µL of each reaction mix into the sample wells of Hard-Shell™ 96-Well PCR Plates, low profile, thin wall, skirted, white/clear (#HSP9601). Refer to the QX Continuum Droplet Digital PCR System Instruction Manual
- e. For the QX700 ddPCR System, load 5 µL of each reaction mix into the RDG16 cartridge. Refer to the QX700 Droplet Digital PCR System Instruction Manual (10000170603) for additional details

Table 5. Reaction setup for the QX200, QX ONE, or QX600 ddPCR System.

Component	Volume per reaction, µL	Final concentration
2x ddPCR Supermix for Probes (No dUTP)	11	1x
20x target primers/ probe (Dye 1)	1.1	1x**
20x reference primers/probe (Dye 2)	1.1	1x**
Restriction enzyme, diluted*	1.1	2–5 U/reaction
DNA sample or water	Variable	≤50 ng†
Total volume	22	—

* This component should be replaced by water if digestion is performed prior to Droplet Digital PCR.

** 900 nM primers/250 nM each probe.

† For most human gDNA experiments, see the loading recommendations in step 1 of this section.

Table 6. Example of a multiplex copy number variation assay on the QX Continuum System.

Component	Volume per reaction, µL	Final concentration
4x ddPCR Supermix for Probes	4.5	1x
20x target 1 primers/probe (Dye 1)	0.9	900 nM/250 nM
20x target 2 primers/probe (Dye 2)	0.9	900 nM/250 nM
20x target 3 primers/probe (Dye 3)	0.9	900 nM/250 nM
20x target 4 primers/probe (Dye 4)	0.9	900 nM/250 nM
Diluted restriction enzyme (see DNA digestion section)	1.125	2–5 units*
Sample	Variable	Up to 16.5 ng/µL**
RNase-/DNase-free water	Variable	—
Total volume	18	—

* For DNA ≤3.3 ng/µL, restriction enzyme digestion may not be necessary, and no incubation is needed for digestion. The reaction mixture can be loaded into the QX Continuum System immediately.

** Maximum input should be ≤264 ng per reaction.

6. For detailed dye information, please refer to Table 1 for fluorophore compatibility for each instrument.

Table 7. Reaction assembly for the QX700 Droplet Digital PCR System.

Component	Final concentration	Volume per reaction, μ L	
		5X Buffer A	10X Buffer A
naica Multiplex ddPCR Mix Buffer A	1x	1.4	0.7
naica Multiplex ddPCR Mix Buffer B	4%*	0.28	0.28
20x Target primers/probe (Dye 1)**	1x	0.35	0.35
20x Reference primers/probe (Dye 2)**	1x	0.35	0.35
Restriction enzyme***	2-5 U/reaction	Variable	Variable
DNA sample	\leq 50 ng/reaction****	Variable	Variable
RNase-/DNase-free water	—	Variable	Variable
Total volume		7	7

*Suggested final concentration not to exceed 5%. Buffer B is provided at an initial concentration of 100%.

**Additional primer/probe sets with different dyes may be added according to Table 1.

***This component should be replaced by water if digestion is performed prior to Droplet Digital PCR.

****For most human gDNA experiments, see the loading recommendations in step 1 of this section.

7. For reaction assembly with the QX700 Droplet Digital PCR System, see Table 7.
 - a. Thaw Buffer A completely before each use. Vortex thoroughly (suggested three times for 5–10 sec each at maximum speed) and briefly centrifuge to collect the liquid at the bottom of the tube.
 - b. For Buffer B, it is recommended to start with a final concentration of 4% and not to exceed 5% during assay optimization. Typical final concentrations range from 2 to 5%.
 - c. Vortex the primers and probes thoroughly before use. After combining all reagents, vortex thoroughly (suggested 5-10 sec at maximum speed) to mix the contents. Centrifuge briefly to collect the liquid at the bottom of the tube before loading the reaction mix onto consumable chips; immediately load the reaction on the respective chip. It is not recommended to freeze the combined reagent solution. For RDG16 Cartridges, the final well reaction volume is 5 μ L.

- For the QX600 Droplet Reader, refer to the QX600 Droplet Reader and QX Manager Software Standard Edition User Guide (10000153877) or the QX600 Droplet Reader and QX Manager Software Premium Edition User Guide (10000153878)
- For the QX ONE ddPCR System, refer to the QX ONE Droplet Digital PCR System and QX ONE Software Instrument Guide (10000116512) and the QX ONE Software User Guide for Standard Edition (10000116655) or Regulatory Edition (10000116656)
- For the QX Continuum ddPCR System, refer to the QX Continuum Droplet Digital PCR System Instruction Manual (10000170603)
- For the QX700 ddPCR System, refer to the QX700 System Analysis Software User Guide (10000171494)

Visit [bio-rad.com/CNV](https://www.bio-rad.com/CNV) for more information.

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Data Acquisition and Analysis

Follow instructions for data acquisition and analysis based on the droplet reader used.

- For the QX200 Droplet Reader, refer to the QX200 Droplet Reader and QX Manager Software Standard Edition User Guide (10000107223) or the QX200 Droplet Reader and QX Manager Software Regulatory Edition User Guide (10000107224)



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