BIO-RAD

ddPCR Multiplex Supermix

Catalog #	Description
12005909	**ddPCR Multiplex Supermix**, 1.2 ml (2 x 0.6 ml vials), 200 x 20 µl reactions
12005910	**ddPCR Multiplex Supermix**, 3 ml (5 x 0.6 ml vials), 500 x 20 µl reactions
12005911	**ddPCR Multiplex Supermix**, 12.5 ml (5 x 2.5 ml vials), 2,500 x 20 µl reactions

For research purposes only.

Description

ddPCR Multiplex Supermix is a 4x concentrated, ready-to-use reaction mix containing all components — except primers, probe(s), and template — required for probe-based Droplet Digital PCR (ddPCR). The hot-start feature of the polymerase in the supermix enables partitioning of sample into droplets while keeping the enzyme inactive at ambient temperature. The supermix has been optimized to provide higher capacity and empower higher-order multiplexing when you use probe-based assays.

Storage and Stability

ddPCR Multiplex Supermix is stable at –20°C through the expiration date printed on the label. It can be stored at 4°C for up to 2 weeks. Repeated freezing and thawing of the supermix is not recommended.

Bio-Rad sells dithiothreitol (DTT; catalog #12012171) separately at a 300 mM concentration. DTT tubes should be thawed only once and can be stored at –20°C for 1 year. After thawing the tube, it can be stored at 4°C for up to 2 weeks.

Quality Control

ddPCR Multiplex Supermix is free of contaminating DNase and RNase. Stringent specifications are maintained to ensure lot-to-lot consistency.

Recommendations for Optimal Results

DNA digestion with a restriction endonuclease that does not cut target or reference amplicon and is insensitive to methylation is recommended for optimal performance and precision of ddPCR Multiplex Supermix (see guidelines in DNA Digestion section).

Required Equipment

The QX200 Droplet Digital PCR System (#1864001), QX200 AutoDG Droplet Digital PCR System (#1864100), or QX ONE Droplet Digital PCR System (#12006536) is required.

Reaction Setup

1. Bring the supermix and samples to room temperature. Mix vigorously by vortexing the tube(s) at **maximum speed (3,200–3,500 rpm) for 15 sec**. It is extremely important to mix the 4x concentrated supermix. This process will ensure its homogeneity and help avoid the formation of a concentration gradient during –20°C storage.
2. Centrifuge briefly to collect contents at the bottom of the tube(s).
3. Prepare DNA samples at the desired concentration before setting up the reaction mix.
4. Prepare reaction mix for the number of reactions needed according to the guidelines in Table 1.
   a. Assemble all required components except the sample.
   b. Dispense equal aliquots into each reaction tube.
   c. Add the sample to each reaction tube as the final step.

Table 1. Example of multiplex copy number variation assay.

	QX200 ddPCR System		QX ONE ddPCR System	
Component	Volume per Reaction, µl	Final Concentration	Volume per Reaction, µl	Final Concentration
ddPCR Multiplex Supermix	5	1x	5	1x
20x target 1 primers/probe (FAM)	1	900 nM/ 250 nM	1	900 nM/ 250 nM
20x target 2 primers/probe (HEX)	1	900 nM/ 250 nM	1	900 nM/ 250 nM
20x target 3 primers/probe (Cy5)	N/A	N/A	1	900 nM/ 250 nM
20x reference 4 primers/probe (Cy5.5)	N/A	N/A	1	900 nM/ 250 nM
Diluted restriction enzyme (see DNA Digestion section)	1	2–5 units	1	2–5 units
300 mM DTT	0.27 (optional)	4 mM	0.27 (required)	4 mM
Sample	Variable	Up to 330 ng	Variable	Up to 330 ng
RNase-/ DNase-free water	Variable	—	Variable	—
Total volume*	**20**	**—**	**20**	**—**

* For the Automated Droplet Generator, prepare 22 µl per reaction.

5. To complete the reaction setup after adding DNA sample, it is extremely important to mix vigorously by vortexing each reaction tube at **maximum speed (3,200–3,500 rpm) for 10 sec**.

6. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes.
7. Allow reaction tubes to equilibrate at room temperature for about 3 min.
8. Transfer the reaction mix from the reaction tubes to the appropriate ddPCR Cartridge as follows:
   - For the QX200 Droplet Digital PCR System, load 20 µl of each reaction mix into a sample well of a DG8 Cartridge. Follow subsequent instructions as specified in the QX200 Droplet Generator Instruction Manual (10031907)
   - For the QX200 AutoDG Droplet Digital PCR System, follow instructions in the Automated Droplet Generator Instruction Manual (10043138)
   - For the QX ONE Droplet Digital PCR System, load 20 µl of each reaction mix into a sample well of a GCR96 Cartridge. Follow subsequent instructions as specified in the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512)

DNA Digestion (recommended)

DNA digestion prior to droplet generation enables optimal accuracy by separating tandem gene copies, reducing sample viscosity, and improving template accessibility for input samples >66 ng per well. Two strategies may be used to perform restriction digestion of DNA samples: digestion directly in the ddPCR reaction during setup or conventional digestion prior to Droplet Digital PCR.

Digestion in ddPCR Reaction

- Dilute the restriction enzyme using the recommended diluent buffer according to the manufacturer's instructions and add 1 µl to the ddPCR reaction as outlined in Table 1
- Approximately 2–5 units of restriction enzyme per 20 µl ddPCR reaction are recommended
- The addition of restriction enzyme buffers with high salt can inhibit Droplet Digital PCR and should be avoided
- HaeIII, MseI, AluI, HindIII, and CviQI have been observed to work well for digestions in ddPCR reactions

Digestion Prior to Droplet Digital PCR

- Use 5–10 enzyme units per microgram DNA and 10–20 enzyme units per microgram genomic DNA
- 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 the ddPCR reaction
- Store digested DNA at –20°C or below
- Prepare the ddPCR reaction mix, as outlined in Table 1, without the diluted restriction enzyme

Thermal Cycling Conditions

Follow instructions based on the system in use:

- For the QX200 Droplet Digital PCR System, carefully transfer droplets into a clean 96-well plate. Seal the plate using the PX1 PCR Plate Sealer (#1814000) at 180°C for 5 sec. Proceed to thermal cycling (see Table 2)
- For the QX200 AutoDG Droplet Digital PCR System, seal the 96-well plate using the PX1 PCR Plate Sealer at 180°C for 5 sec. Proceed to thermal cycling (see Table 2)
- For the QX ONE Droplet Digital PCR System, use appropriate thermal cycling conditions as specified in Table 2 during plate setup. Refer to the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) for plate setup instructions

Table 2. Thermal cycling conditions.*

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		50–65**	1 min***	40
Enzyme deactivation		98	10 min	1
Hold	QX200 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.

** Annealing/extension temperature depends on application type.

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

Data Acquisition and Analysis

Follow instructions based on the system in use:

- For the QX200 Droplet Digital PCR System and the QX200 AutoDG Droplet Digital PCR System, refer to the QX200 Droplet Reader and QuantaSoft Software Instruction Manual (10031906)
- For the QX ONE Droplet Digital PCR System, refer to the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) and the QX ONE Software User Guide for Standard Edition (10000116655) or Regulatory Edition (10000116656)

Visit **bio-rad.com/ddPCRmultiplexsmx** for more information.

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Drive, Hercules, CA 94547
510-741-1000

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