BIO-RAD

ddPCR™ Supermix for Probes

Catalog #	Description
1863026	**ddPCR Supermix for Probes**, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions
1863010	**ddPCR Supermix for Probes**, 5 ml (5 x 1 ml vials), 500 x 20 µl reactions
1863027	**ddPCR Supermix for Probes**, 25 ml (5 x 5 ml vials), 2,500 x 20 µl reactions
1863028	**ddPCR Supermix for Probes**, 50 ml (10 x 5 ml vials), 5,000 x 20 µl reactions

For research purposes only.

Description

ddPCR Supermix for Probes is a 2x concentrated, ready-to-use reaction cocktail containing all components — except primers, probe(s), and template — required for probe-based Droplet Digital™ PCR (ddPCR). The mixture delivers maximum target specificity and fluorescence amplitude and minimum droplet variability to ensure precise target quantification.

The hot-start feature of the polymerase in the supermix enables partitioning of sample into droplets while keeping the enzyme inactive at ambient conditions. The supermix has been optimized to support the amplification and detection of DNA targets using commercially available probe-based assays and is suitable for use with uracil N-glycosylase (UNG) for PCR decontamination. UNG may be purchased from a licensed supplier.

Storage and Stability

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

Quality Control

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

Recommendations for Optimal Results

- Follow general guidelines and recommendations for Droplet Digital PCR (refer to the Droplet Digital PCR Applications Guide, bulletin 6407)
- The concentration of intact human genomic DNA should be ≤66 ng per 20 µl reaction. If using higher concentrations, digest DNA with a restriction endonuclease (see guidelines in DNA Digestion section)

Required Equipment

The QX200™ Droplet Digital PCR System (catalog #1864001), QX200 AutoDG™ Droplet Digital PCR System (#1864100), QX600™ Droplet Digital PCR System (#17007769), QX600 AutoDG Droplet Digital PCR System (#17008371), or QX ONE™ Droplet Digital PCR System (#12006536) is required.

Refer to the QX200 Droplet Reader and QX Manager Software Standard Edition User Guide and QX200 Droplet Generator Instruction Manual (10000107223 and 10031907, respectively), the Automated Droplet Generator Instruction Manual (10043138), or the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) for ordering information about consumables, such as oils, cartridges, gaskets, plates, and seals.

Reaction Setup

1. 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 the tubes.
2. Prepare samples at the desired concentration before setting up the reaction mix.
3. Prepare the reaction mix for the number of reactions needed according to the guidelines in Table 1. Assemble all required components except the sample, dispense equal aliquots into each reaction tube, and add the sample to each reaction tube as the final step.

Table 1. Preparation of the reaction mix.

Component	Volume per Reaction, µl	Final Concentration
2x ddPCR Supermix for Probes	10	1x
20x target primers/probe (FAM, Cy5, or ROX)	1	900 nM/250 nM
20x target primers/probe (HEX/VIC, Cy5.5, or ATTO 590)	1	900 nM/250 nM
Diluted restriction enzyme (see DNA Digestion section)	1	Variable
Sample	Variable	Up to 330 ng*
RNase-/DNase-free water	Variable	—
Total volume**	**20**	**—**

* Sample concentrations >66 ng per reaction and certain applications may require restriction digestion for optimal target detection. If digestion is not required, prepare the ddPCR reaction mix without the diluted restriction enzyme.

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

4. Mix thoroughly by vortexing the tubes. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes. Allow reaction tubes to equilibrate at room temperature for about 3 minutes.
5. Transfer the reaction mix from the reaction tubes to the appropriate ddPCR Cartridge as follows:
   - For the QX600 or 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 QX600 or 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 fragmentation by restriction 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. Choose a restriction endonuclease that does not cut either the target or reference amplicon and that is insensitive to methylation. Four-base cutters and high-fidelity enzymes are preferred.

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 QX600 or 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 180ºC for 5 sec. Proceed to thermal cycling (see Table 2)
- For the QX600 or 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		60	1 min**	40
Enzyme deactivation		98	10 min	1
Hold	QX600 or QX200 ddPCR System	4	30 min	1
	QX ONE ddPCR System	25	1 min	1

* For the PTC Tempo Deepwell Thermal Cycler or C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module, 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.

Data Acquisition and Analysis

Follow instructions based on the system in use:

- For the QX600 Droplet Digital PCR System and the QX600 AutoDG Droplet Digital PCR System, refer to the QX600 Droplet Reader and QX Manager Software Standard Edition User Guide (10000153877)
- For the QX200 Droplet Digital PCR System and the QX200 AutoDG Droplet Digital PCR System, refer to the QX200 Droplet Reader and QX Manager Software Standard Edition User Guide (10000107223)
- 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/ddPCRsmxProbes** for more information.

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