QX700™ ddPCR™ EvaGreen® Supermix

Catalog #	Description
12025290	**QX700 ddPCR EvaGreen® Supermix**, 0.2 mL (2 x 100 µL vials), 200 x 5 µL reactions
12025474	**QX700 ddPCR EvaGreen® Supermix**, 0.5 mL (5 x 100 µL vials), 500 x 5 µL reactions
12025517	**QX700 ddPCR EvaGreen® Supermix**, 2.5 mL (2x 1,250 µL vials), 2,500 x 5 µL reactions

For research purposes only.

Description

QX700 ddPCR EvaGreen® Supermix is a 5x concentrated, ready-to-use reaction cocktail containing all components—except primers and template—required for Droplet Digital PCR™ (ddPCR) on QX700 systems. The mixture delivers maximum target specificity and fluorescence amplitude and minimum droplet variability to ensure precise target quantification. Conventional cycling protocols are used for dye-based detection in ddPCR.

The hot-start feature of the polymerase in the supermix enables partitioning of the sample into droplets while keeping the enzyme inactive at ambient temperatures. The supermix has been optimized to support the amplification and detection of DNA targets using commercially available EvaGreen® Assays.

Storage and Stability

QX700 ddPCR EvaGreen® Supermix is stable at –20°C through the expiration date printed on the label. The Supermix should be stored at –20°C for both short-term use and long-term storage.

Quality Control

QX700 ddPCR EvaGreen® Supermix is free of contaminating DNase. Stringent specifications are maintained to ensure lot-to-lot consistency.

Recommendations for Optimal Results

The concentration of intact human genomic DNA should be ≤16.5 ng per 5 µL reaction. If using higher concentrations (16.5–25 ng per 5 µL reaction), digest the DNA with a restriction endonuclease that does not cut the target or reference amplicons (see guidelines in the DNA Digestion section).

Required Equipment, Software, and Consumables

- QX700E Droplet Digital PCR System* (catalog# 17011036), QX700S Droplet Digital PCR System* (#17010638), or QX700HT Droplet Digital PCR System* (17010628)
- Optimal supermix performance requires QX700 System Control Software v1.5, QX700 System Analysis Software v1.5, and QX700 Supermix Configurator v1.5
- RDG16 Cartridges, Pack of 12 (#12025252)

* For Research Use Only. Not for use in diagnostic procedures.

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
5x QX700 ddPCR EvaGreen® Supermix	1	1x
Forward primer	Variable	100–250 nM
Reverse primer	Variable	100–250 nM
Diluted restriction enzyme (see DNA Digestion section)	1	Variable
DNA template* and RNase-/DNase-free water	Variable	Up to 25 ng**
Total volume	**5**	**—**

* Suggested amplicon length: 60–200 bp, 40-60 %GC.

** Sample concentrations >16.5 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.

4. Mix thoroughly by vortexing the tubes. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes.
5. Once the reaction mixtures are ready, load 5 µL of each reaction mix into a sample chamber of the RDG16 Cartridge.
6. For sample and plate setup instructions, refer to the QX700 Droplet Digital PCR System Instrument Guide (10000171493) and RDG16 Instructions for Use (10000171484) for sample and plate set up instructions.

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 between 16.5 and 25 ng per chamber. Restriction digestion improves overall performance of ddPCR across applications. 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 reaction during setup or conventional digestion prior to the ddPCR.

Digestion in Droplet Digital PCR

- Efficient digestion of sample DNA can be achieved by direct addition of restriction enzymes to the ddPCR reaction
- Preparing a mastermix is recommended to enable pipetting of larger volumes, for better accuracy and reproducibility
- A low-concentration restriction enzyme is recommended for direct use in the mastermix. If dilution of the restriction enzyme is necessary, use the manufacturer-recommended diluent buffer and add 1 µL to the reaction, as specified in Table 1
- Approximately 0.5–1.25 units of restriction enzyme per 5 µL ddPCR reaction is recommended
- The addition of restriction enzyme buffers with high salt can inhibit ddPCR and should be avoided
- HaeIII, MseI, AluI, HindIII, and CviQI have been found to work well for digestions in ddPCR

Digestion Prior to Droplet Digital PCR

- Restriction enzyme digestion can be carried out as a separate reaction before ddPCR setup
- 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 manufacturer
- 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
- Store digested DNA at –20°C or below
- Prepare the ddPCR mix as outlined in Table 1 without the diluted restriction enzyme

Thermal Cycling Conditions

Proceed to thermal cycling (see protocol in Table 2).

Table 2. Thermal cycling conditions.

Cycling Step	Temperature, °C	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	10 min	1°C/sec	1
Denaturation	95	20 sec		40
Annealing/ extension	55–65	30 sec		40

Data Acquisition and Analysis

See Table 3 for recommended exposure times.

Table 3. Recommended Exposure Time

Channel	Color	Times (msec)
FAM	Blue	85
HEX	Teal	273
ROX	Green	365
ATTO-590	Yellow	337
Cy5	Red	51
Cy5.5	Infra-Red	470
ATTO-550	Purple	110

Note: Select the recommended channel colors and exposure times for data acquisition as follows: For Prime PCR SYBR® Green Assays, set the exposure time for the blue channel to 55 msec for data acquisition. If the fluorescence signal reaches ≥60,000 RFU, rescan the plate using a shorter exposure time of 45 msec in the blue channel.

For QX700E Droplet Digital PCR System, QX700S Droplet Digital PCR System, and QX700HT Droplet Digital PCR System, refer to QX700 Droplet Digital PCR System Instrument Guide (10000171493).

Visit **bio-rad.com/QX700EvaGreenSMX** for more information.

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