BIO-RAD

QX200™ ddPCR™ EvaGreen® Supermix

Catalog #	Description
1864033	**QX200™ ddPCR™ EvaGreen Supermix®**, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions
1864034	**QX200™ ddPCR™ EvaGreen Supermix®**, 5 ml (5 x 1 ml vials), 500 x 20 µl reactions
1864035	**QX200™ ddPCR™ EvaGreen Supermix®**, 25 ml (5 x 5 ml vials), 2,500 x 20 µl reactions
1864036	**QX200™ ddPCR™ EvaGreen Supermix®**, 50 ml (10 x 5 ml vials), 5,000 x 20 µl reactions

For research purposes only.

Description

QX200™ ddPCR™ EvaGreen® Supermix is a 2x concentrated, ready-to-use reaction cocktail containing all components — except primers and template — required for Droplet Digital™ PCR (ddPCR). The mixture delivers maximum target specificity and fluorescence amplitude with 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 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 EvaGreen® Assays, and is suitable for use with uracil N-glycosylase (UNG) decontamination protocols. UNG may be purchased from a licensed supplier.

Storage and Stability

QX200™ ddPCR™ EvaGreen® Supermix 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

QX200™ ddPCR™ EvaGreen® Supermix 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 ddPCR (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 that does not cut target or reference amplicons (see guidelines in DNA Digestion section)

Required Equipment

- QX200 Droplet Generator or Automated Droplet Generator (catalog #1864002 or 1864101, respectively)
- QX200 Droplet Reader (catalog #1864003)
- C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module (catalog #1851197)
- PX1™ PCR Plate Sealer (catalog #1814000)

Please refer to the QX200 Instruction Manuals (#10031906 and 10031907) or the Automated Droplet Generator Instruction Manual (#10043138) for ordering information on consumables (oils, cartridges, gaskets, plates, and seals).

Reaction Setup

1. Thaw all components to room temperature. Mix thoroughly by vortexing the tube to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of the tube.
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 QX200™ ddPCR™ EvaGreen® Supermix	10	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 100 ng**
Total volume	**20**	**–**

* Suggested amplicon length: 80–250 bp.
** 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.

4. Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube. Allow reaction tubes to equilibrate at room temperature for about 3 minutes.
5. Once the reaction mixtures are ready, load 20 µl of each reaction mix into a sample well of a DG8™ Cartridge for QX200™/QX100™ Droplet Generator (catalog #1864008) followed by 70 µl of QX200 Droplet Generation Oil for EvaGreen® (catalog #1864005 or 1864006) into the oil wells, according to the QX200 Droplet Generator Instruction Manual (#10031907). For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (#10043138).

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 66 and 100 ng per well. 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 ddPCR reaction during setup, or conventional digestion prior to ddPCR.

Digestion in ddPCR Reaction

- Efficient digestion of sample DNA can be achieved by direct addition of restriction enzymes to the 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 ddPCR and should be avoided
- HaeIII, MseI, AluI, HindIII, and CviQI have been observed to work well for digestions in ddPCR reactions

Digestion Prior to ddPCR

- Restriction enzyme digestion can be carried out as a separate reaction before ddPCR reaction 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
- 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

1. After droplet generation with the QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate, or remove the droplet plate containing ddPCR droplets from the Automated Droplet Generator. Seal the plate with the PX1 PCR Plate Sealer.
2. Proceed to thermal cycling (see protocol in Table 2) and subsequent reading of droplets in the QX200 Droplet Reader.

Table 2. Cycling conditions for Bio-Rad's C1000 Touch Thermal Cycler.*

Cycling Step	Temperature, °C	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	5 min	2°C/sec	1
Denaturation	95	30 sec		40
Annealing/extension	60	1 min		40
Signal stabilization	4	5 min		1
	90	5 min		1
Hold (optional)	4	Infinite		1

* Use a heated lid set to 105°C and set the sample volume to 40 µl.

Data Acquisition and Analysis

1. After thermal cycling, place the sealed 96-well plate in the QX200 Droplet Reader.
2. Open QuantaSoft™ Software to set up a new plate layout according to the experimental design. Refer to the QX200 Droplet Reader and QuantaSoft Software Instruction Manual (#10031906).
3. Under Setup, double click on a well in the plate layout to open the Well Editor dialog box.
4. Designate the sample name, experiment type, **QX200 ddPCR EvaGreen Supermix** as the supermix type, target name, and target type: **Ch1** for FAM.
5. Select **Apply** to load the wells and when finished, select **OK**.
6. Once the plate layout is complete, select **Run** to begin the droplet reading process. Select **EvaGreen** as the dye set used and run options when prompted.
7. After data acquisition, select samples in the well selector under Analyze. Examine the automatic thresholding applied to the 1-D amplitude data and, if necessary, set thresholds or clusters manually.
8. The concentration reported is copies/µl of the final 1x ddPCR reaction.

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

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

D096172 Ver D (10028376) 17-0888 0817