BIO-RAD

QX700™ ddPCR™ Supermix for Residual DNA Quantification

Catalog #	Description
12025342	QX700 ddPCR Supermix for Residual DNA Quantification, 0.2 mL (2 x 0.1 mL vials), 200 x 5 µL reactions
12025523	QX700 ddPCR Supermix for Residual DNA Quantification, 0.2 mL (5 x 0.1 mL vials), 500 x 5 µL reactions

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

Description

QX700 ddPCR Supermix for Residual DNA Quantification is a 5x 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 with minimum droplet variability to ensure precise target quantification for the detection of residual host cell DNA.

The hot-start features of the polymerase enable partitioning of samples into droplets while keeping the enzyme inactive at ambient conditions. The Supermix has been optimized to support the amplification and detection of DNA targets (up to 4-plex) using hydrolysis-probe-based assays, and is compatible with the use of uracil N-glycosylase (UNG) for PCR decontamination. UNG may be purchased from a licensed supplier.

Required Equipment

- QX700 E Droplet Digital PCR System* (catalog #17011036), QX700 S Droplet Digital PCR System* (#17010638), or QX700 HT Droplet Digital PCR System* (#17010628)
- RDG16 Cartridges, pack of 12 (#12025252)
- RDG16 Cartridge Holder (#12025262)

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Storage and Stability

QX700 ddPCR Supermix for Residual DNA Quantification is stable at –20°C through the expiration date printed on the labels. The Supermix remains in a liquid state at –20°C and can be stored at this temperature for routine use.

Quality Control

ddPCR Supermix for Residual DNA Quantification is free of contaminating DNase. Stringent specifications are maintained to ensure lot-to-lot consistency.

Recommendations for Optimal Results

Perform DNA extraction for complex samples with high salt (≥1.0 M salt) or low pH (buffer ≤3.0 pH), and perform proteinase K treatment for samples containing a high concentration of protein (≥0.1 mg/mL) prior to preparing the ddPCR reaction mix to improve amplification. For less complex samples, pretreatment is not necessary if the sample is diluted at least 25-fold.

- Follow general guidelines and recommendations for ddPCR
- Suggested input quantities of DNA sample are 10 fg–85 ng per 5 µL reaction; add femtogram to picogram amounts of DNA for assays targeting highly repetitive sequences, such as long or short interspersed nuclear elements (LINE or SINE), and picogram to nanogram amounts of DNA for assays targeting single-copy gene sequences. A restriction endonuclease digestion is recommended when using higher concentrations of intact genomic DNA (>16.5 ng per 5 µL reaction)
- An assay-specific standard curve is required to convert target copy number to mass concentration for assays targeting an unknown copy number of LINE, SINE, or Alu-like sequences
- A no template control (NTC) should be included to rule out cross-contamination

Reaction Setup

1. Thaw all components at room temperature. Mix thoroughly by vortexing the tubes to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge to collect contents at the bottom of each 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 and dispense equal aliquots into each reaction tube. As the final step, add the sample to each reaction tube.

Table 1. Preparation of the reaction mix.

Component	Volume per Reaction, µL	Final Concentration
5x QX700 ddPCR Supermix for Residual DNA Quantification	1	1x
Target primers/probe	Variable	900 nM/250 nM
Sample	Variable	16.5 ng–25 ng/reaction
RNase-/DNase-free water	Variable	—
Total volume	5	—

*Sample concentrations >16.5 ng/reaction and certain applications may require restriction digestion for optimal target detection. Refer to the DNA digestion section for digestion protocol.

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 an RDG16 cartridge.

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 > 16.5 ng per chamber. 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

- Preparing a mastermix is recommended to enable pipetting of larger volumes, for improved 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 up to 1 µL per 5 µL ddPCR reaction.
- 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

Digestion Prior to Droplet Digital PCR

- Restriction enzyme digestion can be carried out as a separate reaction before ddPCR reaction setup
- Use 5–10 enzyme units per µg DNA and 10–20 enzyme units per µg genomic DNA
- Incubate the reaction for 1 hr at the manufacturer-recommended temperature 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 digested DNA to reduce the salt content of the sample in the ddPCR reaction
- Store digested DNA at –20°C or below

Thermal Cycling Conditions

Proceed to thermal cycling (see protocol in Table 2). Refer to the QX700 Droplet Digital PCR System Instrument Guide (10000171493) for plate setup instructions.

Table 2. Thermal cycling conditions.

Cycling	Temperature, ºC	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	10 min	1ºC/sec	1
Denaturation	95	15 sec		40
Annealing/ extension*	55–60	1 min		

*Annealing/extension temperature and time may require adjustments depending on assay design

Data Acquisition and Analysis

1. Ensure that the exposure time is configured according to the recommended value provided in Table 3 for droplet reading and detection.

Table 3. Recommended exposure time.

Channel	Color	Time, msec
1	Blue	85
2	Teal	273
3	Green	365
4	Yellow	337
5	Red	51
6	Infra-Red	470
7	Purple	110

2. After data acquisition, select samples in the chamber list under Analyze Data. Review the automatic thresholding applied to the 1-D dot plot and, if necessary, manually adjust the threshold using either an individual chamber threshold or a common threshold applied across all chambers to separate positive and negative droplets.

3. The concentration reported is copies/µL of the final 1x ddPCR reaction.

Visit **bio-rad.com/web/ddPCRRDQ** for more information.

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