BIO-RAD

ddPCR™ Supermix for Residual DNA Quantification

Catalog #	Description
186-4037	**ddPCR Supermix for Residual DNA Quantification**, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions
186-4038	**ddPCR Supermix for Residual DNA Quantification**, 5 ml (5 x 1 ml vials), 500 x 20 µl reactions
186-4039	**ddPCR Supermix for Residual DNA Quantification**, 25 ml (5 x 5 ml vials), 2,500 x 20 µl reactions
186-4040	**ddPCR Supermix for Residual DNA Quantification**, 50 ml (10 x 5 ml vials), 5,000 x 20 µl reactions

For research purposes only.

Description

ddPCR Supermix for Residual DNA Quantification 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 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 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.

Storage and Stability

ddPCR Supermix for Residual DNA Quantification is stable at –20°C through the expiration date printed on the labels. 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 Residual DNA Quantification 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)
- Suggested input quantities of DNA sample are 10 fg–100 ng per 20 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
- 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
- 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 and the protein concentration in the final ddPCR reaction is ≤0.1 mg/ml

Required Equipment

- QX100™ or QX200™ Droplet Generator (catalog #186-3002 or 186-4002, respectively) or Automated Droplet Generator (catalog #186-4101)
- QX100 or QX200 Droplet Reader (catalog #186-3003 or 186-4003, respectively)
- C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module (catalog #185-1197)
- PX1™ PCR Plate Sealer (catalog #181-4000)

Please refer to the QX100 or QX200 Instruction Manuals (#10026321 and 10026322 or 10031906 and 10031907, respectively) 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 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, 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 Residual DNA Quantification	10	1x
Target primers/probe	Variable	900 nM/250 nM
Sample	Variable	10 fg–100 ng/reaction
RNase-/DNase-free water	Variable	—
Total volume*	**20**	**—**

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

4. Mix thoroughly by vortexing the tubes and centrifuge briefly to ensure that all components are at the bottom of the reaction tubes.
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 #186-4008) followed by 70 µl of Droplet Generation Oil for Probes (catalog #186-3005) into the oil wells, according to the QX100 or QX200 Droplet Generator Instruction Manual (#10026322 or 10031907, respectively). For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (#10043138).

Thermal Cycling Conditions

1. After droplet generation with the QX100 or 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 QX100 or QX200 Droplet Reader.

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

Cycling	Temperature, °C	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	10 min	2.0°C/sec	1
Denaturation	95	30 sec		40
Annealing/ extension	60	1 min		
Enzyme deactivation	98	10 min		1
Hold (optional)	4	∞		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 QX100 or QX200 Droplet Reader.
2. Open QuantaSoft™ Software to set up a new plate layout according to the experimental design. Refer to the QX100 or QX200 Droplet Reader and QuantaSoft Software Instruction Manual, (#10026321 or 10031906).
3. Under Setup, double click on a well in the plate layout to open the Well Editor dialog box.
4. Designate sample name, **ABS** as the experiment type, **ddPCR Supermix for Probes** as the supermix type, target name, and the appropriate fluorescence channel for the dye used: **Ch1 Unknown** for FAM or **Ch2 Unknown** for HEX or VIC.
5. Select **Apply** to load the wells and, when finished, select **OK**.
6. Once the plate layout is complete, save the template and select **Run** to begin the droplet reading process. Select the appropriate dye set used and the 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 manually by applying either the single well or multiwell threshold between the positive and negative droplets. Use the NTC well to determine the appropriate threshold for sample wells (see Figure 1).
8. The concentration reported is copies/µl of the final 1x ddPCR reaction.

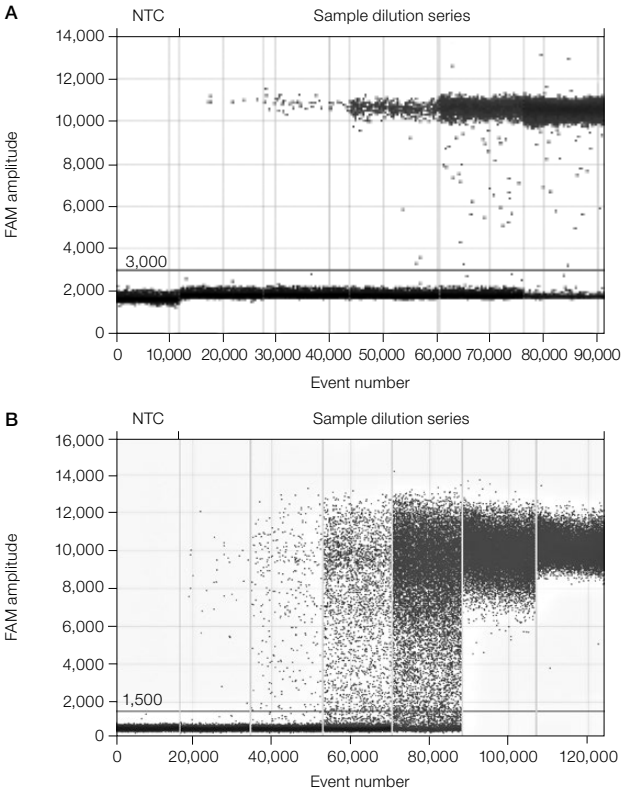

Fig. 1. 1-D fluorescence amplitude plots. A, *Escherichia coli* DNA was serially diluted tenfold and analyzed using Droplet Digital PCR. The 1-D plot shows a multiwell threshold set above the negative droplets in the NTC well. **B**, Chinese hamster ovary (CHO) DNA was serially diluted tenfold and analyzed using Droplet Digital PCR. The 1-D plot shows a multiwell threshold set above the negative droplets in the NTC well.

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

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