BIO-RAD

ddPCR™ Supermix for Residual DNA Quantification

Catalog #	Description
1864037	**ddPCR Supermix for Residual DNA Quantification**, 2 ml (2 x 1 ml vials), 200 x 20 µl reactions
1864038	**ddPCR Supermix for Residual DNA Quantification**, 5 ml (5 x 1 ml vials), 500 x 20 µl reactions
1864039	**ddPCR Supermix for Residual DNA Quantification**, 25 ml (5 x 5 ml vials), 2,500 x 20 µl reactions
1864040	**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 and minimum droplet variability to ensure precise target quantification for the detection of residual host cell DNA.

The hot-start feature of the polymerase in the supermix enables 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

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 the tubes to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge 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 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. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes.
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)

Thermal Cycling Conditions

Follow instructions based on the system in use:

- For the QX600 or QX200 Droplet Digital PCR System, after droplet generation with the QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate. Seal the plate using the PX1 PCR Plate Sealer (#1814000) at 180°C for 5 sec. Proceed to thermal cycling (see Table 2)
- For the QX600 or QX200 AutoDG Droplet Digital PCR System, remove the droplet plate containing ddPCR droplets from the Automated Droplet Generator. Seal the 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, thermal cycling is integrated into and sequentially performed by the system itself. Hence, no additional equipment or sample handling is required for this step. Refer to the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) for plate setup instructions. Use appropriate thermal cycling conditions as specified in Table 2

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		95	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

1. 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)
2. 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.
3. The concentration reported is copies/µl of the final 1x ddPCR reaction.

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

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10000162847 Ver B (10048259) 23-0360 0423