ddPCR™ CHO Residual DNA Quantification Kit

Catalog #	Description
17000031	**ddPCR™ CHO Residual DNA Quantification Kit**, 200 x 20 µl reactions

For research purposes only.

Description

The ddPCR CHO Residual (Chinese hamster ovary) DNA Quantification Kit is designed for the quantification of residual host cell DNA (HCD). HCD carried over during the process of manufacturing therapeutic proteins and vaccines poses safety concerns and must not exceed levels established by regulatory agencies such as the U.S. Food and Drug Administration and the World Health Organization. The ddPCR CHO Residual DNA Quantification assay is a 20x concentrated, ready-to-use primer-probe mix, and the ddPCR Supermix for Residual DNA Quantification is a 2x concentrated, ready-to-use supermix. The kit is compatible with uracil DNA glycosylase (UDG) for use in decontamination procedures.

Storage and Stability

The ddPCR CHO Residual DNA Quantification Assay and the ddPCR Supermix for Residual DNA Quantification included in the kit are stable through the expiration date printed on their labels when stored at –20°C and protected from light. Once thawed, the kit can be stored at 4°C for up to 2 weeks. Repeated freezing and thawing of the kit is not recommended.

Kit Contents

Each kit includes one tube of the 20x assay mix and two tubes of 2x ddPCR Supermix for Residual DNA Quantification, both sufficient for 200 reactions.

Required Equipment

- QX100™ or QX200™ Droplet Generator (catalog #1863002 or 1864002, respectively) or Automated Droplet Generator (catalog #1864101)
- QX100 or QX200 Droplet Reader (catalog #1863003 or 1864003, respectively)
- C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module (catalog #1851197)
- PX1™ PCR Plate Sealer (catalog #1814000)

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 such as oils, cartridges, gaskets, plates, and seals.

Quality Control

The ddPCR CHO Residual DNA Quantification Assay and ddPCR Supermix for Residual DNA Quantification are free of detectable DNase activities and free of detectable *E. coli*, CHO, mouse, human, and yeast DNA. Stringent specifications are maintained to ensure lot-to-lot consistency.

Recommendations for Optimal Results

- Follow the general guidelines and recommendations in the Droplet Digital™ PCR Applications Guide (bulletin 6407)
- Use low DNA binding tubes for all sample dilutions and reaction setup
- Suggested DNA input is 5 fg–5 pg per 20 µl reaction
- Run a cell line-specific standard curve to determine the conversion factor to convert target copy number to mass concentration. Once the conversion factor has been determined, subsequent runs with samples derived from the same cell line do not require standard curves. Ensure that the test samples and samples used to generate the standard curve are handled under the same conditions
- Prepare the standard curve in an area separate from the test sample to reduce the risk of DNA contamination
- Run several no template control (NTC) wells

Sample Preparation Guidelines

One of two strategies may be used to prepare the DNA samples: direct addition of samples to the ddPCR reaction or DNA extraction prior to Droplet Digital PCR.

Direct Addition of Samples in ddPCR Reaction

- The sample can be added directly to the ddPCR reaction if the sample is sufficiently diluted (≥100-fold) prior to adding to the ddPCR reaction
- Up to 0.5 mg/ml IgG can be added directly to the final reaction mix
- Add 5 mM DTT to the reaction if the final reaction contains more than 0.05 mg/ml IgG
- Include DTT in the standard curve if using DTT in test samples to ensure proper calibration

DNA Extraction Prior to Droplet Digital PCR

- Perform DNA extraction for samples with high salt content (≥1.0M salt), low pH (buffer ≤3.0 pH), or high protein content (≥1.0 mg/ml) prior to preparing the ddPCR reaction mix

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 each tube and store protected from light.

2. Prepare samples at the desired concentration before setting up the reaction mix.

3. Prepare the reaction mix at room temperature 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
20x ddPCR CHO Residual DNA Quantification Assay	1	1x
DTT (optional)	Variable	5 mM
DNA sample	Variable	5 fg–5 pg
RNase-/DNase-free water	Variable	–
Total Volume*	**20**	**–**

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

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 min.

5. Once the reaction mixtures are ready, load 20 µl of each reaction mix into a sample well of a DG8™ Cartridge (catalog #1864008) and then load 70 µl of Droplet Generation Oil for Probes (catalog #1863005) 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 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 Step	Temperature, ℃	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	10 min	2°C/sec	1
Denaturation	95	30 sec		5
Annealing/extension	53	1 min		
Denaturation	95	30 sec		40
Annealing/extension	70	1 min		
Hold	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 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, respectively).

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 (No dUTP)** as the supermix type, target name(s), and target type(s): **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 the appropriate dye set and run options when prompted.

7. After data acquisition, select samples in the well selector under Analyze. Examine the automatic threshold 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.

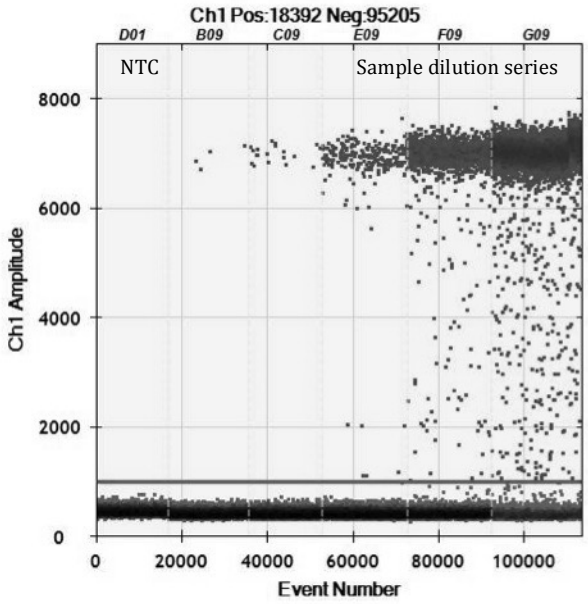
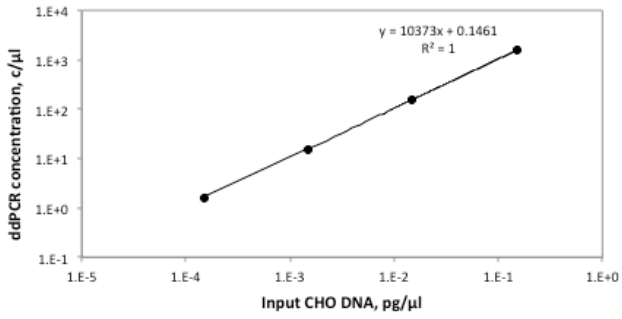


Fig. 1. 1-D fluorescence amplitude plot. A tenfold serial dilution was performed with CHO DNA and analyzed using Droplet Digital PCR. The 1-D plot shows a multiwell threshold set above the negative droplets in the NTC well.

Conversion of copies/µl to pg/µl

1. Run a tenfold serial dilution of CHO DNA standard (0.25 pg/µl to 0.00025 pg/µl) in the final ddPCR mix). Plot the ddPCR concentration data (c/µl) vs. the amount of DNA input (pg/µl).



2. Perform least square linear regression of the data to determine the slope from the regression line.

3. Calculate DNA concentration in an unknown sample using the equation below:

   U = C x (1/slope)

   where

   U = DNA concentration in the unknown sample, pg/µl
   C = ddPCR concentration reported for the unknown, c/µl

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