Quick-Start Protocol September 2022

QIAcuity® CHO resDNA Quant Kit

QIAcuity CHO resDNA Quant Kit consists of QIAcuity CHO resDNA Quant Master Mix (2x), internal control and positive control, and dPCR qualified water. The kit is shipped on dry ice and should be stored protected from light at –30 to –15°C in a constant-temperature freezer upon receipt. Under these conditions the kit components are stable, without showing any reduction in performance and quality, until the expiry date indicated on the label. This kit does not include QIAcuity CHO resDNA Standard Kit (cat. no. 250223). The QIAcuity CHO resDNA Standard Kit is a dPCR-verified absolute quantification standard that can be used in combination with QIAcuity CHO resDNA Quant Kit for validation of quantitation accuracy or bridging studies.

Further information

- *QIAcuity User Manual Extension: QIAcuity Application Guide*: **www.qiagen.com/HB-2839**
- *QIAcuity User Manual:* **www.qiagen.com/HB-2717**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Kit content

Component	Quantity	Cap color
QIAcuity CHO resDNA Quant Master Mix (2x), 500 µl (24 reactions)	4	Red
QIAcuity CHO resDNA Quant Positive Control, lyophilized	1	Green
QIAcuity CHO resDNA Quant Internal Control, lyophilized	1	Yellow
dPCR Qualified Water, 1.8 mL	3	Clear

Notes before starting

To maintain a working environment free of external DNA contamination, we recommend the following precautions for accurate and reproducible dPCR results:

- Wear lab coats, goggles, and gloves throughout the procedure.
- Decontaminate your dPCR workspace and labware (pipets, tube racks, etc.) before each experiment to render any DNA contamination ineffective in dPCR.
- Store sample materials and control templates separately from other reagents. Physically separate dPCR setup workspaces from post-dPCR processing operations.

- Do not remove the QIAcuity nanoplate from its protective sealed bag until immediately before use. Do not remove the sealer foil from previously used QIAcuity nanoplates that releases dPCR product DNA into the air and contaminate results.
- Pipetting accuracy and precision affect the consistency of results. Make sure that no bubbles are pipetted into the wells of the QIAcuity nanoplate. Use sterile filter-tip pipettes.
- At least one No Template Control (NTC) sample should be included in the runs to detect any external DNA contamination.
- DNA samples with ≥20 kb average length should be fragmented by restriction digestion before partitioning. Enzymatic fragmentation of larger DNA ensures even distribution of template throughout the QIAcuity nanoplate.
- The following validated enzymes will not cut within the amplified sequence. It is sufficient to digest DNA templates in 10 min at room temperature (15–25°C) when added directly to the reaction mix at the indicated concentrations.

Validated restriction enzymes

6-cutter restriction enzymes			
*Eco*RI	0.25 U/µL EcoRI-HF®, NEB®	*PvuII*	0.025 U/µL PvuII, NEB
	0.025 U/µL Anza™ 11 EcoRI, Thermo Fisher Scientific (TFS)		0.025 U/µL Anza 52 PvuII, TFS
		XbaI	0.025 U/µL Anza 12 XbaI, TFS

Procedure

Rehydration of the reagents

Component	To be added	Final concentration
QIAcuity CHO resDNA Quant Positive Control	100 µL dPCR Qualified Water	2800 copies/µL (or 784 fg/µL)
QIAcuity CHO resDNA Quant Internal Control	1000 µL dPCR Qualified Water	4000 copies/µL

Vortex and spin briefly after reconstitution. Incubate for 20 min at 37°C.

Reaction setup

1. Thaw the CHO resDNA Quant Master Mix (2x), positive and internal controls, DNA samples and dPCR qualified water. Vigorously mix the CHO resDNA Quant Master Mix and the individual solutions. Centrifuge briefly to collect liquids at the bottom of the tubes.
2. Prepare a reaction mix for the number of reactions needed according to the table hereafter. Due to hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity instrument. The stringency of hot-start, along with other proprietary chemical components in CHO resDNA Quant Master Mix (2x) is essential for delivering highest performance in residual DNA quantification.

Component	Reagent/sample volume Nanoplate 26k (24-well)	Final concentration
QIAcuity CHO resDNA Quant Master Mix (2x)	20 µL	1x
QIAcuity CHO resDNA Quant Internal Control	1 µL (recommended)	100 ± 20 copies/µL*
dPCR Qualified Water	Variable	–
Template DNA† or QIAcuity CHO resDNA Quant Positive Control‡	Variable	–
Restriction enzyme (optional)§	Up to 1 µL	0.025–0.25 U/µL
Total reaction volume	40 µL	–

* Expected dPCR result when 1 µL of internal control is added to the 40 µL reaction volume. It is recommended for maximal precision to add the internal control directly in the master mix.

† Template loading amounts should not exceed 50 pg per reaction. Further dilution of samples is recommended when template loading amounts exceed 50 pg per reaction or when inhibitors are present in the sample. Sample purification is in general not required. For details, please see the *QIAcuity User Manual Extension: Application Guide*.

‡ QIAcuity CHO resDNA Quant Positive Control can be added to the reaction instead of template DNA to confirm PCR reaction conditions are optimal. Positive control loading amounts may vary according to the experimental setup and **should not exceed 19 µL.** We recommend use of 1–5 µL of positive control per reaction (70–350 copies/µL final concentration in reaction).

§ For long gDNA samples with ≥20 kb average length.

3. Vortex gently and spin down the reaction mix.
4. Dispense appropriate volumes of the reaction mix into the wells of a standard PCR plate. Then, add template DNA into each well that contains the reaction mix. Make sure all components are mixed well. Centrifuge briefly.
5. Transfer the content of each well from the standard PCR plate to the wells of the nanoplate avoiding air bubbles.
6. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.

   Note: For exact sealing procedure, please see the *QIAcuity User Manual*.
7. If a restriction enzyme for DNA digestion has been included in the reaction, leave the plate for 10 min at room temperature.

Thermal cycling and imaging conditions

1. Program the cycler of the QIAcuity instrument according to the following table:

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
Two-step cycling (5 cycles)		
Denaturation	15 s	95
Combined annealing/extension	30 s	60
Two-step cycling (40 cycles)		
Denaturation	15 s	95
Combined annealing/extension	30 s	66

2. Recommended Imaging settings:

Target	Detection channel	Exposure/gain
Target assay (CHO)	Green	500/6
Internal Control	Yellow	500/6

3. Place the nanoplate into the QIAcuity instrument and start the dPCR program.
   Note: For details, please see the *QIAcuity User Manual*.

Analysis

1. Use absolute quantification in QIAcuity Software Suite to calculate the target CHO DNA concentration in the reaction in copies/µL. Use following table for calculating residual CHO DNA amounts in fg/µL.

Kit	Target copy number	Amplicon size	Conversion factor (copies/µL to fg/µL)
QIAcuity CHO resDNA Quant Kit	Approx. 1,000,000	<100 bp	0.28

CHO DNA concentration (copies/µL)	CHO DNA concentration (fg/µL)
10	2.8
20	5.6
100	28
1000	280

* Exemplary calculations for converting CHO DNA concentration from copies/µl to fg/µL.
CHO DNA concentration (fg/µL) = CHO DNA concentration (copies/µL) *0.28.

Revision history

Date	Changes
September 2022	Updated the General information, Further information, Kit content, Notes before starting, and Procedure sections to include additional information.