Quick-Start Protocol November 2022

QIAcuity® HEK293 resDNA Quant Kit

QIAcuity HEK293 resDNA Quant Kit consists of QIAcuity HEK293 resDNA Quant Master Mix (2x), internal control and positive control, and dPCR qualified water. The kit is shipped and should be stored protected from light at 2–8°C in a constant-temperature fridge 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 HEK293 resDNA Standard Kit (cat. no. 250225). The QIAcuity HEK293 resDNA Standard Kit is a dPCR-verified absolute quantification standard that can be used in combination with QIAcuity HEK293 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	Storage (°C)
QIAcuity HEK293 resDNA Quant Master Mix , lyophilized (24 reactions)	4	Red	2–8
QIAcuity Quant Rehydration Buffer, 500 µL	4	Orange	2–8
QIAcuity HEK293 resDNA Quant Positive Control, lyophilized	1	Green	2–8
QIAcuity HEK293 resDNA Quant Internal Control, lyophilized	1	Yellow	2–8
dPCR Qualified Water, 2 mL	2	Clear	2–8

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 new 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 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 HEK293 resDNA Quant Positive Control	100 µL dPCR Qualified Water	3400 copies/µL (or 5236 fg/µL)
QIAcuity HEK293 resDNA Quant Internal Control	1000 µL dPCR Qualified Water	4000 copies/µL

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

Component	To be added	Final concentration
QIAcuity HEK293 resDNA Quant Mastermix (lyophized) (24 reactions)	500 µL QIAcuity Quant Rehydration Buffer*	2x

* We recommend reconstitution of 1 tube of lyophilized QIAcuity HEK293 resDNA Quant Mastermix with 1 tube of QIAcuity Quant Rehydration Buffer for each 24 reactions.

Vortex and spin briefly after reconstitution. Proceed to reaction setup.

Note: Upon reconstitution, kit components should be stored at 2–8°C.

Reaction setup

1. Thaw the HEK293 resDNA Quant Master Mix (2x), positive and internal controls, DNA samples and dPCR qualified water. Vigorously mix the HEK293 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 HEK293 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 HEK293 resDNA Quant Master Mix (2x)	20 µL	1x
QIAcuity HEK293 resDNA Quant Internal Control	1 µL (recommended)	100 ± 20 copies/ µL *
dPCR Qualified Water	Variable	–
Template DNA† or QIAcuity HEK293 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.

‡ QIAcuity HEK293 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 10 µL.** We recommend use of 1–5 µL of positive control per reaction (85–425 copies/µL final concentration in reaction).

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

3. Vortex gently and spin down the reaction mix. 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.
4. Transfer the content of each well from the standard PCR plate to the wells of the nanoplate avoiding air bubbles.
5. 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*.
6. 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 (HEK293)	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 HEK293 DNA concentration in the reaction in copies/µL. Use following table for calculating residual HEK293 DNA amounts in fg/µL.

Kit	Target copy number	Amplicon size	Conversion factor (copies/µL to fg/µL)
QIAcuity HEK293 resDNA Quant Kit	Approx. 1,000,000	<100 bp	1.54

HEK293 DNA concentration (copies/µL)	HEK293 DNA concentration (fg/µL)
10	15.4
20	30.8
100	154
200	308
1000	1540

* Exemplary calculations for converting HEK293 DNA concentration from copies/µL to fg/µL.
HEK293 DNA concentration (fg/µL) = HEK293 DNA concentration (copies/µL) * 1.54

Revision history

Date	Changes
August 2022	Corrected the table headers on one of the tables under Analysis section (from "CHO DNA Concentration" to "HEK293 DNA Concentration").
November 2022	Edited according to new brand style guide. Added information in Introduction and Procedure sections.

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For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.