

Quick-Start Protocol

QIAcuity® HEK293 resDNA Sizing Kit

QIAcuity HEK293 resDNA Sizing Kit is designed for quantifying and determining the size of HEK293 or human residual DNA on QIAcuity dPCR System using 2 assays that target different amplicon lengths: a short fragment in the Green Channel and a long fragment in the Crimson Channel. The Internal Control is detected in the Yellow Channel. All assays employ a ready-to-use primer–probe mix.

Store reagents at –30°C to –15°C in a constant-temperature freezer away from light.

Further information

- QIAcuity HEK293 DNA Sizing Tool: www.qiagen.com/HEK293-sizing
- QIAcuity HEK293 resDNA Sizing Kit Handbook: www.qiagen.com/HB-1021
- QIAcuity User Manual: www.qiagen.com/HB-2717
- QIAcuity Application Guide: www.qiagen.com/HB-2839
- Safety Data Sheets: www.qiagen.com/safety

Important points before starting

- Wear laboratory coats, goggles, and gloves throughout the procedure.
- Decontaminate all dPCR workspaces and labware (including pipettes, tube racks, etc.) prior to each experiment to mitigate potential DNA contamination in dPCR assays.
- Store sample materials and control templates separately from other reagents. Ensure physical separation of dPCR setup areas from post-dPCR processing operations.
- Do not remove QIAcuity Nanoplate from its protective sealed bag until immediately before use. Avoid removing the sealer foil from previously used QIAcuity Nanoplates to prevent the release of dPCR product DNA into the environment, which may compromise results.
- A fluorescent dye is provided as a component of QIAcuity resDNA Quant Master Mix for reliable detection of proper filling in the dPCR plates.
- Pipetting accuracy and precision affect the consistency of quantification results. Make sure that no air bubbles are introduced into the wells of the dPCR Nanoplates during pipetting.
- DNA samples with an average length of ≥ 20 kb should undergo fragmentation by restriction digestion prior to partitioning. Enzymatic fragmentation of large DNA templates facilitates uniform distribution across QIAcuity Nanoplate.

- The validated enzyme (*Xba*I) employed will not cleave into the amplified sequence. To perform DNA template digestion, add the enzyme directly to the reaction mix and incubate for 10 min at room temperature (15–25°C) at the recommended concentrations.
- If necessary, thaw kit components and mix thoroughly before use.

Procedure

1. Prepare a reaction mix with all components except the template as shown in Table 1. Hot start means that samples do not need to be kept on ice during setup or when programming QIAcuity.

Note: Prepare 10% extra volume to ensure enough amount for transferring to the Nanoplates.
2. Dispense the reaction mix into a standard PCR plate, then add template DNA to each well.

Note: Refer to the *QIAcuity Application Guide* (www.qiagen.com/HB-2839) for recommended volumes, as they may vary.
3. Transfer the contents from each well of the standard PCR plate into the corresponding wells of a 26k Nanoplate.
4. Seal the Nanoplate properly using QIAcuity Nanoplate Seal provided in QIAcuity Nanoplate kits. For sealing instructions, see the *QIAcuity User Manual* (www.qiagen.com/HB-2717).
5. If *Xba*I is used, incubate the plate for 10 min at room temperature.

Table 1. Reaction setup for Nanoplate 26k (24-well)

Component	Volume (µL) per reaction	Final concentration
4x QIAcuity resDNA Quant Master Mix	10	1x
20x QIAcuity HEK293 resDNA Sizing Assay (Green Channel and Crimson Channel)	2	1x
10x QN IC Probe Assay* (Yellow Channel)	2	0.5x
Restriction Enzyme (<i>Xba</i> I 10 U/µL)	0.1	0.025 U/µL
QN Internal Control DNA dPCR*	0.16	~1000 copies/µL
Template DNA or HEK Positive Control†	Variable	–
RNase-free Water	Variable	–
Total reaction volume	40	–

* Add 10x QN IC Probe Assay and QN Internal Control DNA dPCR for a multiplex reaction to include an Internal Control.

† For HEK Positive Control the use of 2 µL input volume per reaction is recommended (~1000 copies/µL final concentration).

Thermal cycling and imaging conditions

1. Set the cycling conditions (Table 2) and imaging settings (Table 3) under the dPCR parameters in QIAcuity Software Suite or QIAcuity instrument.
2. Place the Nanoplate into QIAcuity instrument and start the dPCR program.

Table 2. Cycling conditions

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

Table 3. Imaging settings*

Channel	Exposure (ms)	Gain
Green	500	6
Yellow	500	6
Crimson	300	4

* Imaging settings might need to be adjusted according to the assay. Always start with the recommended settings.

Data analysis

1. To set up a plate layout according to the experimental design, open QIAcuity Software Suite and define the reaction mixes, samples, and controls. Plate layout can be defined before or after the nanoplate run.
Note: Refer to the *QIAcuity User Manual* for details about setting up the plate layout. After the nanoplate run, the raw data are automatically sent to QIAcuity Software Suite.
2. For data analysis, open QIAcuity Software Suite and select one nanoplate for analysis in Plate Overview of the Software Suite.
Note: Refer to the *QIAcuity Application Guide* and *QIAcuity User Manual* for details on how to analyze the data to get absolute quantification data.

Sizing and concentration analysis

For details on how to import and analyze the data in QIAcuity HEK293 DNA DNA Sizing Tool, refer to *QIAcuity HEK293 resDNA Sizing Kit Handbook* (www.qiagen.com/HB-1021).

Table 4. Converting output concentrations

Target copy number (copies per genome)	Amplicon sizes (bp)	Conversion factor (copies/μL to pg/μL)
>20	~83 (short)	0.129
>20	~345 (long)	0.143

Table 5. Examples of converting HEK293 DNA concentrations from copies/μL to pg/μL

HEK293 DNA concentration (copies/μL)	HEK293 DNA mass concentration	
	Short amplicon (pg/μL)	Long amplicon (pg/μL)
10	1.29	1.43
20	2.58	2.86
100	12.9	14.3
1000	129	143

Document Revision History

Date	Description
01/2026	Initial release



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