June 2026

Quick-Start Protocol

QIAcuity® *P. pastoris* resDNA Quant Kit

The QIAcuity *P. pastoris* resDNA Quant Kit (cat. no. 250329) is designed to detect residual *Pichia pastoris* DNA on the QIAcuity dPCR System using an assay in the Green 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, protected from light, in a constant-temperature freezer.

Further information

- *QIAcuity P. pastoris resDNA Quant Kit Handbook*: **resources.qiagen.com/250329**
- *P. pastoris resDNA Quant Standard Kit Quick-Start Protocol*: **resources.qiagen.com/HB-3838**
- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- *QIAcuity Application Guide*: **www.qiagen.com/HB-2839**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Important points before starting

- Wear laboratory coats, goggles, and gloves throughout the procedure.
- Decontaminate all dPCR workspaces and labware (including pipettes, tube racks, etc.) before 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 the 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 before partitioning. Enzymatic fragmentation of large DNA templates facilitates uniform distribution across the QIAcuity Nanoplate.
- The validated enzyme (*PvuII*) 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 Sample or QIAcuity *P. pastoris* resDNA Quant Postive Control (PC) 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 Sample or QIAcuity *P. pastoris* resDNA Quant PC to each well.

   Note: Refer to the *QIAcuity Application Guide* 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*.
5. If restriction enzyme *PvuII* 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
4× QIAcuity resDNA Quant Master Mix	10	1×
20x QIAcuity resDNA *P. pastoris* Assay [Green Channel]	2	1×
10× QN IC Probe Assay* [Yellow Channel]	2	0.5×
Restriction Enzyme (*PvuII* 10 U/µL)	0.1	0.025 U/µL
QN Internal Control DNA dPCR*	0.16	~1000 copies/µL
Sample or QIAcuity *P. pastoris* resDNA Quant Positive Control†	Variable	–
RNase-free Water	Variable	–
Total reaction volume	**40**	–

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

† For QIAcuity *P. pastoris* resDNA Quant 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

* 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.

Concentration analysis

1. For conversion of cp/µL to fg/µL the conversion factor and examples in Table 4 and Table 5 should be used.
2. For details on how to analyze the data, refer to *QIAcuity P. pastoris resDNA Quant Kit Handbook*.

Table 4. Converting output concentrations

Target copy number (copies per genome)	Amplicon sizes (bp)	Conversion factor (copies/µL to fg/µL)
Multicopy	<100	0.379

Table 5. Examples of converting *P. pastoris* DNA concentrations from copies/µL to fg/µL

P. pastoris DNA concentration (copies/µL)	*P. pastoris* DNA mass concentration (fg/µL)
10	3.79
20	7.58
100	37.9
1000	379

Document Revision History

Date	Changes
06/2026	Initial release.

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