Quick-Start Protocol

February 2025

QIAcuity® RCL Quant Kit

This protocol is optimized for the detection of Replication Competent Lentivirus (RCL) using the VSV-G Assay and an Internal Control with the QIAcuity MasterMix in multiplex reactions on the QIAcuity digital PCR (dPCR) instrument.

The VSV-G Assay target is detected in the Green Channel, while the Internal Control is detected in the Yellow Channel. Both assays come in a ready-to-use primer–probe mix.

Upon receipt, store the kit reagents protected from light at –30°C to –15°C in a constant-temperature freezer for long-term storage.

Further information

- *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**

Notes before starting

- Always start with the cycling conditions and primer concentrations specified in this protocol.
- A fluorescent reference dye is provided as a component of the QIAcuity MasterMix for reliable detection of proper partition filling in the dPCR plates.

- Pipetting accuracy and precision affect the consistency of results. Make sure that no air bubbles are introduced into the wells of the dPCR plate during pipetting.
- Thaw kit components, if required. Mix all kit components before use.

Procedure

Reaction setup

1. Prepare a reaction mix, which contains all components except the template, according to Table 1 on the facing page. Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity.

   Note: It is recommended to prepare a 10% surplus to safely transfer the needed volume to the nanoplates.

2. Dispense appropriate volumes of the reaction mix into the wells of a standard PCR plate. Add template DNA into each well that contains the reaction mix.

   Note: The appropriate amounts of reaction mix and template DNA depend on various parameters. Refer to the *QIAcuity Application Guide* for details.

3. Transfer the contents of each well of the standard PCR plate to the wells of a 26k nanoplate.
4. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate kits. For sealing instructions, see the *QIAcuity User Manual*.

Thermal cycling and imaging conditions

5. Set the cycling conditions under the dPCR parameters in the QIAcuity Software Suite or at the QIAcuity instrument according to Tables 2 and 3, next page.

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

Component	Volume per reaction	Final concentration
4x QIAcuity MasterMix	10 µL	1x
20x QIAcuity RCL (VSV-G) Assay [Green Channel]	2 µL	1x
10x QN IC Probe Assay* [Yellow Channel]	2 µL	0.5x
Restriction Enzyme (*Pvu*II 10 U/µL)	0.1 µL	0.025 U/µL
RNase-free water	Variable	
Template DNA§ or RCL Positive Control‡	Variable	
QN Internal Control DNA dPCR*	0.16 µL	1000 cop/µL
Total reaction volume	**40 µL**	–

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

‡ For RCL Positive Control the use of 2 µL input volume per reaction is recommended (~1000 cop/µL final concentration).

§ Samples with template gDNA amounts higher than 10 µg/reaction of total DNA should be diluted prior to testing.

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 (FAM)	500	6
Yellow (HEX)	500	6

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

6. For multiple probe detection, activate the appropriate channel and deactivate the other channels in Imaging, under the dPCR parameters in the QIAcuity Software Suite or the QIAcuity instrument.

   Always start with the recommended imaging settings in Table 3.
7. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

Data analysis

1. To set up a plate layout according to the experimental design, open the 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 the QIAcuity Software Suite.
2. For data analysis, open the 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.

Document Revision History

Date	Description
02/2025	Initial release

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