Quick-Start Protocol

April 2025

CGT Lentivirus Lysis Kit

This protocol is optimized for the processing of lentiviral vectors for genome titer quantification on the QIAcuity® digital PCR instrument using the CGT Lentivirus Lysis Kit (cat. nos. 250323, 250324). The kits are optimized for usage with the QIAcuity OneStep Advanced Probe Kit (cat. nos. 250131, 250132) and the QIAcuity Cell and Gene Therapy (CGT) dPCR Assays (**qiagen.com/qiacuity-cgt**).

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

- Lentiviral vector (LVV) samples with a high expected titer (e.g., titer > 1 x 10^8 vg/mL) or samples inhibiting DNase I may be diluted using the CGT PBS buffer before setting up the DNase I digest.
- Long-term storage of LVV samples or lysates is not recommended.
- Reconstitute lyophilized DNase I enzyme: Dissolve lyophilized DNase I (1500 Kunitz units) in 550 µL water. To avoid loss, do not open the vial. Inject water into the vial using an RNase-free needle and syringe. Mix gently by inverting and do not vortex.
- For long-term storage of DNase I, remove the stock solution from the glass vial and divide it into single-use aliquots. Aliquots can be stored at –15°C to –30°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not freeze the aliquots after thawing.

Sample processing and reaction setup

1. Thaw the viral vector samples at room temperature or, alternatively, on ice (2–8°C) right before use.
2. Prepare DNase I digest reaction mix according to Table 1. Spin down and mix properly by pipetting up and down 5 times or by flicking the tube 5 times. Spin down and incubate for 30 min at 37°C (e.g., on a thermal cycler). Afterwards, cool down the reaction at 4°C for 5 min. Spin down and mix properly by pipetting up and down 5 times or by flicking the tube 5 times before proceeding to the next step.

   Important: Do not skip even if removal of residual DNA contaminants in the sample has already taken place in the upstream sample preparation. However, in this case, the DNase I enzyme can be replaced by nuclease-free water.

Table 1. DNase I reaction setup

Component	Volume/reaction
Lentiviral Vector Sample	1–8 µL
Buffer RDD	5 µL
DNase I	5 µL
Nuclease-free water	12 µL
Total reaction volume (add CGT PBS buffer)	**50 µL***

* Total reaction volume can be scaled up or scaled down to 20 µL. When scaling up, the number of reactions of the kits will decrease accordingly.

3. Prepare an LVV lysis mixture according to Table 2. Spin down and mix thoroughly by vortexing the reaction mix 5 times, 1 s each. Spin down and incubate for 10 min at 95°C (e.g., in a thermal cycler). After incubation, cool down for 5 min at 4°C. Spin down and proceed to the next step. Long-term storage of the lysate is not recommended.

Table 2. LVV lysis reaction setup

Component	Volume/reaction
Viral Vector sample (from step 2)	4 µL
CGT Dilution Buffer	34 µL
Proteinase K	2 µL
Total reaction volume	**40 µL***

* Total reaction volume can be scaled up or scaled down to 20 µL.

4. Prepare the dPCR reaction mix using the QIAcuity OneStep Advanced Probe Kit in a standard 96-well PCR plate according to Table 3 below. Seal plate and mix thoroughly by vortexing the reaction mix 5 times, 1 s each, spin down.
5. Transfer the appropriate volume to a nanoplate. Seal nanoplate and load into the QIAcuity instrument. Start run.

Table 3. Reaction setup

	Volume per reaction	
Component	**Nanoplate 26k (24-well)**	**Nanoplate 8.5k (24-well and 96-well)**
4x OneStep Advanced Probe Master Mix	10 µL	3 µL
20x dPCR CGT Assay 1*	2 µL	0.6 µL
Additional CGT Assays (2, 3, 4, 5) for multiplex (optional)†	2 µL	0.6 µL
100x OneStep Advanced RT Mix (Reverse Transcription)	0.4 µL	0.12 µL
Lysate (from step 3)	4–20 µL‡	1.2–6 µL‡
Nuclease-free water	variable	variable
Total reaction volume	**40 µL**	**12 µL**

* Custom designed assays can be used. Start with recommended primers and probe concentrations of 0.8 µM of each primer and 0.4 µM probe.

† Add additional 20x dPCR CGT Assays or gene of interest assays for a multiplex reaction to detect multiple targets at once.

Important: Dye combinations must be different from those used in the 20x dPCR CGT Assay 1. For dye recommendations and the corresponding probe and channels available on the QIAcuity, see the *QIAcuity User Manual* (**qiagen.com/HB-2717**) or the *QIAcuity Application Guide* (**qiagen.com/2839**).

‡ Lysate volume is variable depending on required dilution.

Thermal cycling and imaging conditions

1. Set the cycling conditions under the dPCR parameters in the QIAcuity Software Suite or at the QIAcuity instrument according to Table 4 on the next page.
2. Under the dPCR parameters in the QIAcuity Software Suite or on the QIAcuity instrument, activate all needed channels in Imaging. Start with the imaging settings in Table 5.
3. Place the Nanoplate into the QIAcuity instrument and start the dPCR program.

Table 4. Cycling conditions

Step	Time	Temperature (°C)
Reverse transcription	40 min	50
Initial heat activation	2 min	95
2-step cycling (40 cycles)		
Denaturation	5 s	95
Combined annealing/extension	30 s	60*

* Temperature during annealing/extension and number of cycles might vary depending on assay type. For the CGT dPCR assays, 60°C is the optimum.

Table 5. Imaging settings*

Channel	Exposure (ms)	Gain
Green (FAM)	500	6
Yellow (HEX)	500	6
Crimson (Cy5)	400	8
Orange	400	6
Red	300	4

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

Document Revision History

Date	Description
04/2025	Initial release