Quick-Start Protocol March 2023

CGT Viral Vector Lysis Kit

This protocol is optimized for the processing of viral vectors for genome titer quantification on the QIAcuity® digital PCR instrument using the dPCR CGT Viral Vector Lysis Kits (cat. nos. 250272, 250273). The kits are optimized for usage with the QIAcuity Probe PCR Kit (cat. nos. 250101, 250102, 250103) and the QIAcuity Cell and Gene Therapy (CGT) dPCR Assays (**www.qiagen.com/qiacuity-dpcr-assays**).

The CGT Viral Vector Lysis Kits are shipped at ambient temperature (Box 1) and on dry ice (Box 2). Please store the components of Box 1 at room temperature (15–25°C) and the components of Box 2 at –20°C. The DNase I vials in Box 1 should be stored immediately upon receipt at 2–8°C.

Further information

- *CGT Viral Vector Lysis Kit Handbook*: **www.qiagen.com/HB-3362**
- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- QIAcuity User Manual Extension: Application Guide: **www.qiagen.com/HB-2839**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Viral vector samples with a high expected titer (e.g., titer > 1 x 10^{11} vg/mL) may be diluted using the corresponding storage buffer supplemented with 0.01 % CGT Sample Stabilizer before setup of the DNase I digest. Alternatively, sample dilutions can be performed in protein low binding tubes.
- Long-term storage of AAV samples or lysates is not recommended.
- Dilute the CGT Sample Stabilizer stock (10% (v/v)) 1:10 in nuclease-free water to obtain a 1% (v/v) working solution. Mix thoroughly before use.
- Mix the CGT Lysis Buffer by carefully inverting 3 times before use.
- *Hpa*II restriction enzyme is not included in this kit and must be separately ordered from e.g., Thermo Fisher Scientific (Invitrogen™ ANZA™ 93 *Hpa*II or Thermo Scientific *Hpa*II)
- Proteinase K is not included in this kit. We highly recommend using the following enzymes: RP107B-1 or RP107B-5.

Procedure

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. DNase I digest is directly followed by addition of Proteinase K.

2a. Prepare a DNase I digest reaction mix according to Table 1. Spin down and mix properly by pipetting 5 times up and down 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: Even if removal of residual DNA contaminants in the sample has already taken place in the upstream sample preparation, this step should not be skipped. However, in this case, the DNase I enzyme can be replaced by Nuclease-Free Water.

Table 1. DNase I reaction setup

Component	Volume/reaction
Viral Vector sample (e.g., AAV2)	5 µL
CGT DNase I Buffer (7x)	7.14 µL
DNase I	5 µL
CGT Sample Stabilizer (1%)	5 µL
Nuclease-Free Water	27.86 µL
Total reaction volume	**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.

2b. Following incubation of the DNase I digest, add 2 µL Proteinase K per 50 µL reaction from step 2a. 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 room temperature. Mix thoroughly by vortexing the reaction mix 5 times for 1 s and spin down before proceeding to step 3.

3. Prepare a viral vector lysis mixture according to Table 2 (next page). 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.

   Note: Viral vector samples with an expected high titer can be serially diluted as needed using the CGT Lysis Buffer before incubation at 95°C. Appropriate dilution steps depend on the expected vector titers. Recommended detection range in the dPCR using an 8.5K nanoplate is between 2.5 cop/µL to 15,000 cop/µL.

4. Dilute the lysates from step 3 according to Table 3 (next page). Spin down and mix thoroughly by vortexing 5 times, 1 s each. Spin down and proceed to step 5.

   Note: Viral vector samples with an expected high titer can be diluted using the CGT Dilution Buffer after lysis. The lysates **must** be diluted at least 1:100 taking together step 4 and step 5. A 1:200 dilution is recommended.

Example: A viral vector sample is being diluted 1:20 using the CGT Dilution Buffer in step 4. A 1:10 dilution is recommended in the following step. A 1:5 dilution would be mandatory. Higher dilutions can be performed without concerns.

Table 2. Viral vector lysis reaction setup

Component	Volume/reaction
Viral Vector sample (from step 2b)	5 µL
CGT Lysis Buffer	45 µL
Total reaction volume	**50 µL***

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

Table 3. Lysate dilution setup

Component	Volume/reaction
Viral Vector sample (from step 3)	2.5 µL
CGT Dilution Buffer	47.5 µL
Total reaction volume	**50 µL***

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

5. Prepare the dPCR reaction mix using the QIAcuity Probe PCR Kit according to Table 4 (next page) in a standard 96-well PCR plate. Seal plate and mix thoroughly by vortexing the reaction mix 5 times, 1 s each, spin down and incubate for 10 min at room temperature.

   Important: Sample dilutions can be performed in step 1, step 3, step 4, and step 5. The lysates resulting from step 3 **must** be diluted in total at least 1:100 taking together step 4 and step 5. A 1:200 dilution is recommended.

6. Resuspend the dPCR mix and transfer the appropriate volume to a nanoplate. Seal nanoplate and load into the QIAcuity instrument. Start run.

Thermal cycling and imaging conditions

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

Table 4. dPCR reaction setup

Component	Nanoplate 8.5k (24-well, 96-well)	Nanoplate 26k (24-well)
4x QIAcuity Probe PCR Master Mix	3 µL	10 µL
CGT dPCR Assay, 20x†	0.6 µL	2 µL
Nuclease-Free Water	7.05 µL	23.5 µL
Restriction enzyme *Hpa*II (10 U/µL)	0.15 µL (0.125 U/µL)‡	0.5 µL (0.125 U/µL)‡
Lysate (from step 4)	1.2 µL*	4 µL*
Total reaction volume	**12 µL**	**40 µL**

* Lysate volume is variable depending on required dilution.

† Custom designed assays can be used. Please start with recommended primers and probe concentrations of 0.8 µM of each primer and 0.4 µM probe.

‡ Best performance can be achieved when using the Invitrogen ANZA 93 *Hpa*II enzyme within a range of 0.025–0.125 U/µL or the Thermo Scientific *Hpa*II enzyme within a range of 0.25–0.5 U/µL.

Table 5. Cycling conditions

Step	Time	Temperature [°C]
Initial heat activation	2 min	95
2-step cycling (40 cycles)		
Denaturation	**15 s**	**95**
Annealing/Extension	**30 s**	**60***

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

Document Revision History

Date	Changes
03/2023	Initial release

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