June 2025

CGT Lentivirus Lysis Kits and CGT dPCR Assay Handbook

For determination of lentiviral vector genome titers using a QIAcuity® dPCR system

Sample to Insight

Table of Contents

Kit Contents

CGT Lentivirus Lysis Kit Catalog no. No. of reactions	250323 100	250324 1000*
Proteinase K	0.25 mL	2 x 1.25 mL
DNase I, RNase-free	1 vial	10 vials
CGT PBS Buffer	20 mL	4 x 20 mL
Buffer RDD	2 mL	4 x 2 mL
CGT Dilution Buffer	40 mL	2 x 40 mL
Nuclease-free Water	1.9 mL	1 x 50 mL

* The number of reactions is calculated based on 50 µL DNase I and 40 µL Lysis reactions with Proteinase K as described in this handbook. Increasing reaction volumes reduces number of reactions.

Kit Catalog no. No. of reactions	(20x) 250230–250256; 250300–250321 500*
CGT dPCR Assay, lyophilized	1 vial

* The number of reactions is calculated based on 12 µL QIAcuity dPCR reactions.

Shipping and Storage

The CGT Lentivirus Lysis Kits are shipped at 2–8°C. The kit box should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. The DNase I vials, Buffer RDD, and CGT PBS Buffer should be stored immediately upon receipt at 2–8°C. Under these conditions, the components are stable until expiry date printed on their labels without showing any reduction in performance and quality, unless otherwise indicated on the labels.

The CGT dPCR Assays are shipped at ambient temperature and should be stored protected from light upon receipt at –30°C to –15°C in a constant-temperature freezer for long-term storage (e.g., 12 months). Under these conditions, CGT dPCR Assay Kits are stable without showing any reduction in performance and quality. After reconstitution, the assays are stable for at least 12 months. It is recommended to store the CGT dPCR Assays in aliquots at –30°C to –15°C to avoid repeated freeze–thaw cycles.

Intended Use

The CGT Lentivirus Lysis Kits and CGT dPCR Assay Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of CGT Lentivirus Lysis Kits and CGT dPCR Assay Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The CGT Lentivirus Lysis Kit offers a complete standardized workflow from lysis of lentivirus (LV) samples to quantification of the viral titers. It combines robust sample processing and quantification without the need for laborious purification. The kit is particularly suited to LV samples of different purities (e.g., from in-process samples such as supernatant samples to highly purified lentiviral vector samples).

The CGT Lentivirus Lysis Kit can be used in combination with custom developed assays on the QIAcuity dPCR platforms, qPCR, or other dPCR platforms. However, to obtain optimal quantification results, the use of CGT dPCR Assays in combination with the QIAcuity OneStep Advanced Probe Kit and the QIAcuity dPCR system is highly recommended, offering an end-to-end dPCR workflow comparable to qPCR, but delivering an absolute quantification of genome copies of lentiviral particle samples.

The CGT Lentivirus Lysis Kit, with its unique formula, provides a consistent, accurate, precise, and highly repeatable determination of the final viral titer.

Principle and procedure

The CGT Lentivirus Lysis Kits, together with the CGT dPCR Assays and the QIAcuity instrument, form a unique system for lentiviral vector titer determination that offers the best combination of performance and ease-of-use.

The sample processing and lentiviral titer determination comprises only 3 steps in which DNA impurities such as residual host cell DNA or plasmids are removed, lentiviral vectors are lysed, and raw lysates are finally quantified via dPCR (Figure 1).

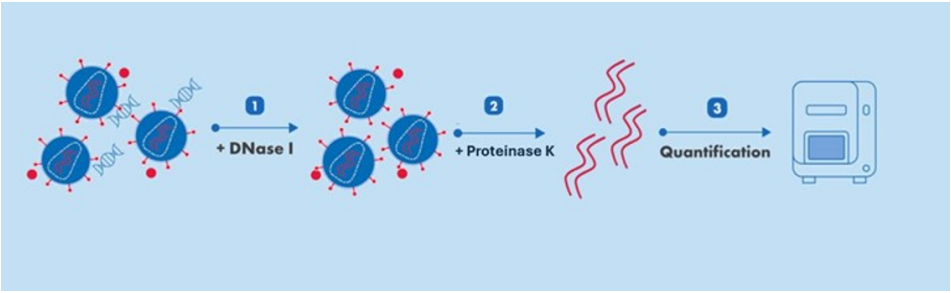

Figure 1. Schematic overview of LV sample processing and titer determination using QIAGEN CGT Lentivirus Lysis Kit and CGT dPCR Assays. (1) DNA impurity removal: eliminate DNA impurities, including host cell DNA and plasmids. **(2)** Vector genome extraction: extract the vector genome through lysis and Proteinase K digestion. **(3)** Precise vector titer determination: use digital PCR to accurately measure vector titers, targeting the gene of interest, LV-specific targets, or backbone assays.

Removal of DNA impurities

DNA impurities, such as plasmids used to produce viral vectors, may affect the final determined titer. The DNase I reaction efficiently removes impurities and prepares the viral particles for subsequent lysis.

Lentivirus particle lysis

The CGT Dilution Buffer and the Proteinase K included in the CGT Lentivirus Lysis Kits are optimized for efficient lentivirus lysis with a wide range of lentivirus input concentrations and a high compatibility with the QIAcuity OneStep Advanced Probe chemistry.

QIAcuity OneStep Advanced Probe chemistry

The hot-start reverse transcription technology and the hot-start QuantiNova DNA polymerase enzyme, along with other proprietary chemical components, enable optimal lentiviral RNA titer determination.

CGT dPCR Assays

Assays are provided in a ready-to-use 20x primer–probe mix, available in up to 3 fluorophore choices (FAM, HEX, or Cy5). The assays, with their double-quenched probe technology, enable singleplexed and multiplexed viral vector titer determination.

Description of protocols

This handbook contains a protocol for processing of lentiviral vector (LVV) particles and a protocol for titer determination using the QIAcuity dPCR technology.

LVV particle processing including Proteinase K

Description of the lentiviral particle processing workflow from removal of DNA impurities to lysed particles ready to be quantified via dPCR. In this protocol **Proteinase K is added** to the particle lysis step.

Absolute quantification of lentiviral vectors using CGT dPCR Assays

Description of the viral RNA genome quantification using the QIAcuity dPCR system.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs), available from the product supplier.

- CGT dPCR assays (cat. nos. 250230–250256; 250300–250321); optional
- QIAcuity OneStep Advanced Probe Kit for viral particles with an RNA genome (cat. nos. 250131 or 250132)
- QIAcuity Nanoplates (cat. nos. 250001, 250011, or 250021)
- Microcentrifuge tubes or PCR plates or strip tubes with appropriate sealing foil. Compatibility of tubes/plates with heating devices needed.
- Single-channel or multichannel pipettor (manual or automatic) with nuclease-free, aerosol-barrier pipette tips
- Heating block, PCR cycler, or water bath capable of reaching 95°C
- Vortexer
- Centrifuge for tubes and plates

Important Notes

Starting material

The CGT Lentivirus Lysis Kits are optimized for processing of LV particles. The amount of starting material can vary greatly, depending on upstream processes (production system, purification, and enrichment of particles). LV samples can be stored in various storage buffers. The processing and quantification workflow is very robust towards various buffer components (e.g., detergents, high salt, carrier) as well as against potential inhibitors (such as coating agents) and secreted intracellular and extracellular material carried over after particle extraction from producer cell lines (such as HEK 293 cells).

High and low titer viral vectors

A wide range of particle titers can be processed using the CGT Lentivirus Lysis Kits. Particles with a high expected titer can be diluted before transfer into the DNase I digest and/or throughout the whole processing workflow (for details, see "Appendix B: Recommendations on Dilution Steps throughout the Processing Workflow Using the CGT Lentivirus Lysis Kit" on page 29).

Genome titers

LV vector genome concentrations can vary depending on the protocol, target location on the genome, DNA impurities, and the intactness of the genome. The use of a multiplex dPCR approach provides a high-resolution characterization of the lentiviral vector genomes. Lentiviral titers of samples determined using qPCR may significantly deviate from viral titers obtained using dPCR due to, for example, the use of standard curves in qPCR.

Protocol: Lentiviral Particle Processing with the Use of DNase I

This protocol is optimized for processing of lentiviral (LVV) particles of different purities for vector genome titer determination using a QIAcuity dPCR system.

Important points before starting

- The Lentivirus Lysis Kit has been optimized for processing of LVV particles.
- LVV particles of different titers can be processed following the same protocol. Dilution series must be adjusted to fit into the dPCR concentration range accordingly. Predilution of the sample in PBS may be necessary for the functionality of the DNase. Dilution should be tested for different sample types. Including a no RT (reverse transcriptase) control is recommend to ensure optimal Dnase I activity with your samples and to identify the optimal dilution range.
- Dilution of lentivirus vector samples can be performed before DNase I treatment or after the lentiviral particle lysis step. However, when diluting before DNase I treatment, the CGT PBS Buffer or standard 1x PBS must be used. Dilutions after lentiviral particle lysis step should be performed using the CGT Dilution Buffer.

Things to do before starting

- Concentrated or DNase-inhibiting viral vector samples may be diluted using standard 1x PBS before starting with the processing workflow described below.

- 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.
- Please make sure that the Buffer RDD and the CGT PBS Buffer are thawed completely without any precipitates before using it. If precipitates are visible, the buffers should be slightly heated.
- Thaw kit components before starting the particle processing and mix all components, also stored at room temperature, right before use.
- Preheat a thermal cycler, heating block, or a water bath to 37°C for the DNase I digest and to 95°C for capsid lysis.

*Kunitz units are commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per mL at 25°C, pH 5.0, with polymerized DNA as the substrate.

Procedure

1. Thaw the viral vector samples at room temperature or, alternatively, on ice (2–8°C) right before use.
2. Prepare a DNase I digest reaction mix according to Table 1 below.
   a. Mix first the viral vector and the CGT PBS Buffer, then add the Nuclease-free Water.
   b. Add the Buffer RDD and the DNase I last. Spin down and mix properly by pipetting 5 times up and down or by flicking the tube 5 times. Do not vortex. Spin down and incubate for 30 min at 37°C (e.g., on a thermal cycler).
   c. Cool down the reaction at 4°C for 5 min.
   d. Spin down and mix properly by pipetting 5 times up and down or by flicking the tube 5 times before proceeding to the next step (Do not vortex!).

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 the Nuclease-free Water.

Table 1. DNase I reaction setup

Component	Volume per reaction (µL)*
Lentiviral Vector sample†	1–8
Nuclease-free Water	12
Buffer RDD	5
DNase I	5
Total reaction volume (add CGT PBS Buffer)	**50**

* 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.

† It may be necessary to dilute the sample in CGT PBS Buffer to ensure that the DNase reaction is working.

3. Prepare a viral vector lysis mixture according to Table 2 below.

    a. Mix first the viral vector sample (from step 2) and CGT Dilution Buffer, then add the Proteinase K last.

    b. Spin down and mix thoroughly by vortexing the reaction mix 5 times, 1 s each.

    c. 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.

    d. Spin down and proceed to the next step.

    Long-term storage of the lysate is not recommended.

Table 2. Viral vector lysis reaction setup

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

* 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.

4. The lysate from Table 2 can be used directly to prepare the PCR reaction mix using the QIAcuity OneStep Advanced Probe Kit according to the protocol "Absolute Quantification of RNA Viral Vectors Using CGT dPCR Assays" on the next page.

    Note: After the lentivirus lysis step, lysates with an expected high titer can be serially diluted as needed using the CGT Dilution Buffer before dPCR.

    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.

Protocol: Absolute Quantification of RNA Viral Vectors Using CGT dPCR Assays

This protocol describes how to set up a QIAcuity PCR for an RNA virus vector genome titer determination of lentiviral vector lysates generated using the CGT Lentivirus Lysis and CGT dPCR Assays.

Important points before starting

- The reverse transcriptase must transcribe the viral RNA to DNA. The 4x QIAcuity OneStep Advanced Probe Kit contains the QuantiNova DNA polymerase, which is inactive at room temperature. Therefore, the PCR protocol must start with a mandatory initial incubation step of 40 min at 50°C for reverse transcription, followed by 2 min at 95°C to activate the enzyme.
- A fluorescent dye is provided as a component of the QIAcuity OneStep Advanced Probe Kit for reliable detection of proper filling in the dPCR plates.
- Always start with the cycling conditions and primer concentrations specified in this handbook.
- CGT dPCR Assays can be used in singleplex or in multiplex reactions.
- 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.

Thing to do before starting

- Thaw the QIAcuity OneStep Advanced Probe Master Mix and mix properly.

Procedure

1. Prepare the PCR reaction mix using the QIAcuity OneStep Advanced Probe Kit according to Table 3 below in a standard PCR plate. Seal plate and mix thoroughly by vortexing the reaction mix 5 times, 1 s each.

   Note: It is recommended to prepare a 10% surplus to completely transfer the required volume to the nanoplate.

Table 3. Reaction setup

	Volume per reaction (µL)	
Component	**Nanoplate 26k (24-well)**	**Nanoplate 8.5k (24-well and 96-well)**
4x OneStep Advanced Probe Master Mix	10	3
20x dPCR CGT Assay 1*	2	0.6
Additional CGT Assays (2, 3, 4, 5) for multiplex (optional)†	2	0.6
100x OneStep Advanced RT Mix (Reverse Transcription)	0.4	0.12
Lysate (from step 3 on page 17)	4 ‡	1.2‡
Nuclease-free water	variable	variable
Total reaction volume	**40**	**12**

* 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* (**www.qiagen.com/HB-2717**) or the *QIAcuity Application Guide* (**www.qiagen.com/2839**).

‡ Lysate volume is variable depending on required dilution.

2. Prepare and mix the PCR reaction and transfer the appropriate volume to the nanoplate. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits and load into the QIAcuity instrument. Start the run.

Thermal cycling and imaging conditions

3. Set the cycling conditions under the dPCR parameters in the QIAcuity Software Suite or the QIAcuity instrument according to Table 4 below.
4. 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 on the facing page.
5. 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*

* Always start with recommended cycling conditions. 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.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center (**www.qiagen.com/FAQ/FAQList.aspx**). The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
DNase I treatment	
Insufficient removal of DNA impurities	Ensure activity of DNase I enzyme. Correct storage of DNase I enzyme after reconstitution: For long-term storage, 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 freezing. Physical denaturation of the DNase I enzyme: Do not vortex the reconstituted DNase I or the DNase I digest reaction. Mix by pipetting, inverting, or flicking. Suboptimal buffer conditions for efficient DNase digest. Make sure to pipette the indicated amount of Buffer RDD without air bubbles. Saturated DNase I digest: Try diluting LVV sample (e.g., 1:10) in CGT PBS Buffer before setting up the DNase I digest.
Reaction setup	Buffer RDD must be used for the DNase I reaction. The buffer composition has been optimized for removal of DNA impurities in LVV samples. Use of alternative buffers may lead to preliminary LV particle opening and viral RNA genome loss. Cool-down at 4°C is required after incubation at 37°C.
Sample storage after DNase I treatment	Sample storage after DNase I treatment is not recommended. The RNase enzymes activity may not be inactivated at this step and is still able to digest accessible RNA. Sample freezing and thawing may lead to LV particle opening and digestion of the viral genome by the active RNase enzymes.

	Comments and suggestions
LV particle lysis	
Insufficient LV particle lysis	DNase I step not performed before entering lysis step: DNase I reaction should not be skipped. The buffer composition of particles is essential for optimal lysis. Addition of DNase I enzyme to the reaction can be skipped. Addition of the Buffer RDD into the DNase I reaction is essential. Incubation at 95°C for 10 minutes skipped: Heat incubation must be performed for efficient LV particle lysis and optimal accessibility of genome targets to be amplified during PCR.
Proteinase K	95°C incubation for 10 minutes skipped: Heat step must be performed for Proteinase K inactivation. Insufficient inactivation leads to the digestion of the polymerase in the PCR step.
Reaction setup	Cool-down at 4°C is required after incubation at 95°C. Deviation from protocol may lead to misquantification of the viral genome titer.
Serial dilution of lysed capsids	
Dilution performed in water	Dilution in water will negatively affect robustness and repeatability of titer determination. The CGT Dilution Buffer formula has been optimized to provide sample stability and high compatibility with the QIAcuity One Step Advanced Probe PCR chemistry.
Higher dilutions needed	Dilutions higher than 200x needed to fit into dPCR concentration range: Higher dilutions are not critical.
PCR	
Lower titer than expected	Lower quantification can be caused by storage of samples which were not measured directly after the LV particle lysis.

	Comments and suggestions
No positive partitions	Sample input below LOD. Sample with low titer or too highly diluted: Try reducing dilution steps. Increase sample input into PCR. Move from 8.5K nanoplates to 26K nanoplates. Assays: Check compatibility with samples. Cycling conditions: Check and optimize annealing/extension temperature with custom-designed assays or assays from other suppliers. Please follow recommended cycling program when using QIAGEN assays. No RT step: Ensure, that the reverse transcriptase step is included in the cycling protocol. Imaging setup: Check channel choice and match with probe dyes. Prolonged initial denaturation: Ensure to run the PCR using the QIAcuity OneStep Advanced Probe PCR mix with an initial denaturation of 2 minutes at 95°C after the RT step. Prolonged denaturation might negatively affect PCR performance.
Infinity signal / no negative partitions	Sample dilution: Increase dilution to fit into the dPCR concentration range. DNase I inactivation: DNase I enzyme not properly inactivated. Please follow instructions described in this handbook.
Poor separation of positive and negative partitions	Custom assays: Please review choice of probe system (quenchers and fluorophores). Follow design guidelines in the appendix of this handbook. Proteinase K inactivation: Proper inactivation of the Proteinase K enzyme is required. Residual Proteinase K activity negatively affects PCR performance and the signal-to-noise ratio. Follow instructions described in the Proteinase K protocol in this handbook to ensure proper Proteinase K inactivation.
Titer	
Titer higher than expected	Insufficient DNase I treatment (see troubleshooting section "DNase I treatment" on page 22). Underestimation of titer with reference method (e.g., conversion from qPCR to dPCR; titer provided by supplier of reference standards not accurate).

	Comments and suggestions
Titer lower than expected	Sample handling: Dilution of LVV particles in water may lead to particle damage and loss of quantifiable genome during downstream processing. Insufficient LV particle lysis (see troubleshooting section “Insufficient LV particle lysis” on page 23 under “LV particle lysis”). Insufficient DNase I inactivation (incubation at 95°C for 10 minutes). Poor PCR/assay performance (see troubleshooting section “PCR” on page 23).
Inconsistent titer determination	See troubleshooting “Handling and storage”, next section.
Two assays lead to different titer	LV vector genome concentrations can vary depending on the protocol, target location on the genome, RNA secondary structures, DNA impurities, and the integrity of the genome. The use of a multiplex dPCR approach provides a high-resolution characterization of the lentiviral vector genomes. Sample of interest might contain fragmented genomes or mispackaged genomes. PCR assays do not perfectly match vector genome (e.g., SNPs in binding sites of primers and/or probes). Poor assay performance: see troubleshooting section “PCR” and design guidelines summarized in the appendix of this handbook.
Handling and storage	
Inconsistent results	Make sure to mix reagents such as the Buffer RDD before each use. Lack of homogeneity might lead to inconsistent results. Avoid repeated freeze-thaw cycles of kit reagents. Poor repeatability between different operators: Ensure operators are following the CGT Lentivirus Lysis Kits protocols. When using automatic pipettors, identical pipetting settings should be used by all operators (e.g., pre-dispense mode recommended over direct pipetting mode).
Provided reagents in the kit not sufficient	DNase I: provided DNase I is sufficient for a total of 100 or 1000 reactions (50 µL reactions). Proteinase K: provided Proteinase K is sufficient for a total of 100 or 1000 reactions (40 µL reactions).
Lid temperature when using a PCR cycler for heating steps	Heated lid (if possible) preferred over unheated lid. The lid temperature should be chosen 5°C higher than the incubation temperature.

	Comments and suggestions
Storage	(Long-term) storage of intermediate workflow products not recommended.
Pipetting volume	Ensure following lower and higher limit of your pipette. We recommend not to pipette volumes lower than 1 µL. Take viscosity of reagents into consideration and adjust pipetting setup accordingly.

Appendix A: Transfer of qPCR Assays to dPCR and Design Guidance for Custom Designed Assays

The CGT Lentivirus Lysis Kit is optimized for use with the CGT dPCR assays. However, the kit can also be used with custom designed assays or assays that have already been successfully used for titer determination via qPCR.

Important factors for success in dPCR include the design of optimal primer pairs and probes, the use of appropriate primer and probe concentrations, and the correct storage of primers and probes. It is particularly important to minimize nonspecific annealing of primers and probes. When designing assays the following aspects should be followed.

T_m of primers and hydrolysis probes

- The T_m of all primers should be 58–62°C and within 2°C of each other.
- The T_m of probes should be 8–10°C higher than the T_m of the primers.
- Avoid a guanidine at the 5′ end of probes, next to the reporter, due to quenching effects.
- Avoid repetitions of 4 or more of the same nucleotide, especially of guanidine.
- Choose the binding strand so that the probe has more C than G bases.

Primer sequence

- Primers should have a length of 18–30 nucleotides with a GC content of 30–70%.
- Primer specificity should always be checked by performing a BLAST search. Ensure that primer sequences are unique within the sample.
- Primer and probes should not be complementary to each other.

- Avoid complementarity of 2 or 3 bases at the 3′ ends of primer pairs to minimize primer–dimer formation.

Storage

- Lyophilized primers and probes should be solved in a small volume of low-salt buffer to yield a concentrated stock solution (e.g., 100 µM). It is recommended to use Buffer TE (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) for standard primers and probes labeled with most fluorescent dyes. Probes labeled with fluorescent dyes such as Cy5 and Cy5.5 should be stored in Buffer TE, pH 7.0.
- Primers and probes should be stored in small aliquots at –20°C. Avoid repeated freeze–thaw cycles.

Transfer of qPCR assays to dPCR

- All rules for proper real-time PCR assay design apply to dPCR.
- Care should be taken such that the recommended cycling conditions and primer–probe concentrations for dPCR are selected.
- For optimization of suboptimal performing assays, a temperature gradient during the annealing steps can be run on any real-time PCR instrument using the QIAcuity OneStep Advanced Probe PCR chemistry.

Additional information can be found in the *QIAcuity Application Guide*: **www.qiagen.com/HB-2839**

Appendix B: Recommendations on Dilution Steps throughout the Processing Workflow Using the CGT Lentivirus Lysis Kit

The CGT Lentivirus Lysis Kit allows processing of viral vector samples of different titers. The kit allows to dilute at different steps throughout the workflow.

Required dilutions throughout the processing workflow

- DNase I reaction

  Viral vector samples can be directly added to the DNase I reaction or must be diluted in the CGT PBS Buffer or standard 1x PBS for some samples reaction without initial dilution. Samples are diluted 1:6.25 in this step. This results in a total dilution of 6.25x or higher.

- LV particle lysis step

  After the DNase I treatment, samples are diluted 1:10 in the CGT Dilution Buffer and Proteinase K. This results in a total dilution of 62.5x taking step 2 and step 3 together.

- dPCR setup

  In the dPCR reaction, the samples are diluted at least 1:10 (for 1.2 µL sample input). This results in a total dilution of 625x, taking steps 2, 3, and 4 together.

Table 6. Overview of total dilutions

Step	Dilution within step	Total dilution
DNase I reaction	1:6.25	6.25x
Optional Predilution, e.g., 1:10	1:62.5	62.5x
LV particle lysis step	1:10	62.5x
With optional Predilution, e.g., 1:10 (from DNase I reaction step)	1:10	625x
Step 4: dPCR setup	1:10	625x
With optional Predilution, e.g., 1:10 (from LV particle lysis step)	1:10	6250x

Appendix C: Dilution Guide

Table 7 shows typical lentiviral titers with exemplary dilutions and expected dPCR copy/µL results.

Table 7. Overview copies per µL

Expected titer (viral particles/mL)	Predilution factor	cop/µL in predilution/ input	cop/µL in DNase step (8 µL sample input)	cop/µL in lysis	cop/µL in dPCR	Lysate input 12 µL dPCR reaction
1.0×10^{11}	100	2.0×10^{6}	3.2×10^{5}	3.2×10^{4}	3200.0	1.2 µL
1.0×10^{10}	10	2.0×10^{6}	3.2×10^{5}	3.2×10^{4}	3200.0	1.2 µL
1.0×10^{9}	1	2.0×10^{6}	3.2×10^{5}	3.2×10^{4}	3200.0	1.2 µL
1.0×10^{8}	1	2.0×10^{5}	3.2×10^{4}	3.2×10^{3}	320.0	1.2 µL
1.0×10^{7}	1	2.0×10^{4}	3.2×10^{3}	3.2×10^{2}	160.0	6 µL
1.0×10^{6}	1	2.0×10^{3}	3.2×10^{2}	3.2×10^{1}	16.0	6 µL

Ordering Information

Product	Contents	Cat. no.
CGT Lentivirus Lysis Kit (100)	For 100 DNase I (50 µL) and Lysis reactions with Proteinase K (40 µL): Proteinase K, DNase I, CGT PBS Buffer, Buffer RDD, CGT Dilution Buffer and Nuclease-free water	250323
CGT Lentivirus Lysis Kit (1000)	For 1000 DNase I (50 µL) and Lysis reactions with Proteinase K (40 µL): Proteinase K, DNase I, CGT PBS Buffer, Buffer RDD, CGT Dilution Buffer and Nuclease-free water	250324
dPCR CGT Assay Albumin (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250300, 250301, 250302
dPCR CGT Assay RPP30 (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250303, 250304, 250305
dPCR CGT Assay RPL32 (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250306, 250307, 250308
dPCR CGT Assay PuroR (FAM, HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250309, 250310
dPCR CGT Assay KanR/NeoR (FAM, HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250311, 250312
dPCR CGT Assay Psi (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250313, 25014, 250315
dPCR CGT Assay RRE (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250316, 25017, 250318
dPCR CGT Assay 5' LTR (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250319, 25020, 250321

Product	Contents	Cat. no.
dPCR CGT Assay ITR2/5 (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250230, 250231, 250232
dPCR CGT Assay bGH polyA (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250233, 250234, 250235
dPCR CGT Assay GFP (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250236, 250237, 250238
dPCR CGT Assay WPRE (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250239, 250240, 250241
dPCR CGT Assay SV40 promoter (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250242, 250243, 250244
dPCR CGT Assay SV40 polyA (FAM, HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250245, 250246
dPCR CGT Assay CMV promoter (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250247, 250248, 250249
dPCR CGT Assay hGH polyA (FAM, HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250250, 250251
dPCR CGT Assay CMV enhancer (FAM, HEX, Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250252, 250253, 250254
dPCR CGT Assay AMP resistance (FAM, HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250255, 250256
QIAcuity OneStep Advanced Probe Kit (1 mL, 5 mL)	Ready-to-use 4x concentrated Master Mix, water	250131, 250132
QIAcuity Nanoplate 8.5K 96-well (10)	10 QIAcuity Nanoplates 8.5K with 96 wells 11 Nanoplate Seals	250021

Product	Contents	Cat. no.
QIAcuity Nanoplate 8.5K 24-well (10)	10 QIAcuity Nanoplates 8.5K with 24 wells 11 Nanoplate Seals	250011
QIAcuity Nanoplate 26K 24-well (10)	10 QIAcuity Nanoplates 26K with 24 wells 11 Nanoplate Seals	250001
QIAcuity One, 2plex Instrument	One-plate digital PCR instrument for detecting up to 2 fluorescent dyes, roller, USB flash memory and QIAcuity Software Suite: includes 1 preventive maintenance visit. 1 year warranty on labor, travel, and parts also included.	911000
QIAcuity One, 5plex Instrument	One-plate digital PCR instrument for detecting up to 5 fluorescent dyes, roller, USB flash memory and QIAcuity Software Suite: includes 1 preventive maintenance visit. 1 year warranty on labor, travel, and parts also included.	911020
QIAcuity Four Instrument	Four-plate digital PCR instrument for detecting up to 5 fluorescent dyes, notebook computer, barcode scanner, roller, USB flash memory and QIAcuity Software Suite: includes installation, training, and 1 preventive maintenance visit. 1 year warranty on labor, travel, and parts.	911040
QIAcuity Eight Instrument	Eight-plate digital PCR instrument for detecting up to 5 fluorescent dyes, notebook computer, barcode scanner, nanoplate roller, USB flash memory and QIAcuity Software Suite: includes installation, training, and 1 preventive maintenance visit. 1 year warranty on labor, travel, and parts.	911050
Related Products		
Nanoplate Seals (11)	11 Nanoplate Seals	250099
RNase-Free DNase Set (50)	1500 Kunitz units RNase-free DNase I	79254
RNase-Free DNase Set (250)	5 x 1500 Kunitz units RNase-free DNase I	79256
QIAcuity Residual DNA Quantification Kits	QIAcuity *E. coli* / CHO / HEK293 resDNA Quant Master Mix (4x) lyophilized, Positive Control, Internal Control, RNAse-Free Water, and respective Standard Kits	250220–250225
CGT Viral Vector Lysis Kit (100)	For 100 DNase I reactions (50 µL): CGT Sample Stabilizer, CGT DNase I Buffer, DNase I, CGT Lysis Buffer, CGT Dilution Buffer, and Nuclease-free Water	250272

Product	Contents	Cat. no.
CGT Viral Vector Lysis Kit (1000)	For 1000 DNase I reactions (50 µL): CGT Sample Stabilizer, CGT DNase I Buffer, DNase I, CGT Lysis Buffer, CGT Dilution Buffer, and Nuclease-free Water	250273
QIAcuity Mycoplasma Quant Kit	For 96 reactions: OneStep Advanced Probe Master Mix (4x), OneStep RT Mix, Positive Control, Internal Control, OneStep Enhancer GC, RNAse-free Water	250261
QIAcuity Mycoplasma CFU Standards Kits	For the detection of frequently occurring mycoplasma contaminants (*Mycoplasma orale*, *Mycoplasma gallisepticum*, or others). QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control, lyophilized	250262–250271
QIAcuity RCL Quant Kit	For 96 reactions: QIAcuity MasterMix, QN Internal Control DNA dPCR, QN IC Probe Assay, VSV-G Assay, Positive Control, RNase-free water	250322

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Document Revision History

Date	Description
06/2025	Initial release

Limited License Agreement for CGT Lentivirus Lysis Kits and CGT dPCR Assay

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