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CGT Viral Vector Lysis Kits and CGT dPCR Assay Handbook

For determination of viral vector genome titers using a QIAcuity dPCR system

Sample to Insight

Contents

Kit Contents 4
Shipping and Storage 5
Intended Use 5
Safety Information 6
Quality Control 6
Introduction 7
Principle and procedure 7
Description of protocols 9
Equipment and Reagents to Be Supplied by User 10
Important Notes 11
Starting material 11
High and low titer viral vectors 11
Genome titers 11
ITR secondary structures 12
Protocol: AAV particle processing without use of Proteinase K 13
Protocol: AAV particle processing using Proteinase K 18
Protocol: Absolute quantification of viral vectors using CGT dPCR Assays 23
Troubleshooting Guide 27
Contact Information 32
Appendix: Transfer of qPCR assays to dPCR and design guidance for custom designed assays 33

Appendix: Recommendations on dilution steps throughout the processing workflow using the CGT Viral Vector Lysis Kit 35
Ordering Information 38
Document Revision History 45

Kit Contents

CGT Viral Vector Lysis Kits Catalog numbers Number of reactions	(Box 1 & Box 2) 250272 100*	(Box 1 & Box 2) 250273 1000*
CGT Sample Stabilizer	0.5 mL	0.5 mL
DNase I, RNase free	1 vial	10 vials
CGT Lysis Buffer	45 mL	4 x 45 mL
CGT DNase I Buffer	1.5 mL	5 x 1.5 mL
CGT Dilution Buffer	40 mL	5 x 40 mL
Nuclease-Free Water	50 mL	4 x 50 mL

* The number of reactions is calculated based on 50 µL DNase I reactions as described in this handbook. Increasing reaction volumes reduces number of reactions.

Kit Catalog no. Number of reactions	(20x) 250230 - 250256 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 Viral Vector Lysis kits are shipped in 2 separate boxes. Box 1 is shipped at ambient temperature and Box 2 is shipped on dry ice.

Box 1 should be stored at room temperature (15–25°C) immediately upon receipt and Box 2 at −30 to −15°C in a constant temperature freezer. The DNase I vials in Box 1 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 upon receipt be stored protected from light at −30 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 to −15°C to avoid repeated freeze-thaw cycles.

Intended Use

The CGT Viral Vector 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 Viral Vector Lysis Kits and CGT dPCR Assay Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The CGT Viral Vector Lysis Kit offers a complete standardized workflow from lysis of Adeno-Associated Virus (AAV) 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 AAV samples of different purities (e.g., from in-process samples such as harvest samples to highly purified viral vector samples).

The CGT Viral Vector 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 Probe PCR 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 encapsidated genome copies of various viral particle samples.

The CGT Viral Vector Lysis Kit, with its improved formula, provides a consistent, accurate, precise, and highly repeatable determination of the final viral titer with or without the need for Proteinase K (Prot-K).

Principle and procedure

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

The sample processing and viral titer determination comprises only 4 steps in which DNA impurities such as residual host cell DNA or plasmids are removed, viral vector capsids are lysed, raw lysates are serially diluted and finally quantified via dPCR (Figure 1).

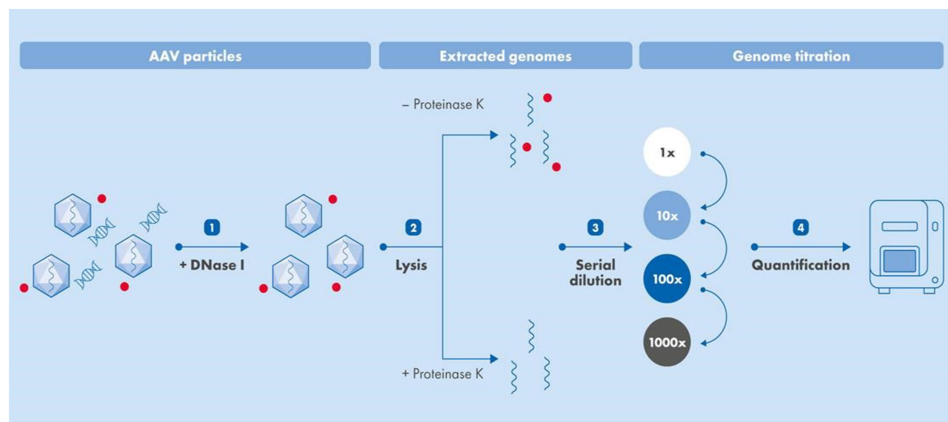

Figure 1. AAV vector titer determination following the CGT Viral Vector Lysis Kit workflow. 1: AAV particles extracted from producer cells (independent of purity) are treated with DNase I for removal of DNA impurities. **2**: Vector genomes are extracted via lysis. Lysis is not Proteinase K dependent. **3**: Extracted genomes are serially diluted into the dPCR concentration range. **4**: Titer determination using the QIAcuity Probe Mix and CGT dPCR Assays.

Removal of DNA impurities

DNA impurities such as plasmids used to produce viral vectors and DNA originating from damaged particles might have an impact on the final determined titer. Removal of impurities is required to enable safe and reliable dosing. The DNase I reaction efficiently removes impurities and prepares the viral particles for subsequent capsid lysis.

Viral Vector Capsid lysis

The CGT Lysis Buffer included in the CGT Viral Vector Lysis Kits is optimized for efficient capsid lysis (independent of AAV serotypes) with a wide range of capsid (of various purities) input concentrations and a high compatibility with the QIAcuity Probe PCR chemistry.

QIAcuity Probe PCR chemistry

The hot-start QuantiNova DNA polymerase enzyme, along with other proprietary chemical components, enables optimal viral titer determination.

CGT dPCR Assays

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

Description of protocols

This handbook contains 2 types of protocols for processing of viral vector particles (particularly AAV particles) and 1 protocol for titer determination using the QIAcuity dPCR technology.

AAV particle processing without the use of Proteinase K

Description of the viral particle processing workflow from removal of DNA impurities to lysed particles ready to be quantified via dPCR. Capsid lysis is achieved **without the need of Proteinase K.**

AAV particle processing including Proteinase K

Description of the viral 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 capsid lysis step.

Absolute quantification of viral vectors using CGT dPCR Assays

Description of the viral 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, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Proteinase K (RP107B-1 or RP107B-5); only for Proteinase K protocol
- Restriction enzyme *Hpa*II (Thermo Scientific™ *Hpa*II (10 U/µL, cat. nos. ER0511, ER0512) or Invitrogen™ Anza™ 93 *Hpa*II (cat.no. IVGN0936))
- CGT dPCR assays (cat. nos. 250230 – 250256); optional
- QIAcuity Probe PCR Kit for viral particles with a DNA genome (cat. nos. 250101-250103)
- 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 Viral Vector Lysis Kits are optimized for processing of AAV particles. The amount of starting material can vary greatly depending on upstream processes (production system, purification and enrichment of particles). AAV samples can be stored in various storage buffers. The processing and quantification workflow is very robust towards various buffer components (detergents, high salt, carrier) as well as against potential inhibitors such as coating agents, 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 Viral Vector 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.

Genome titers

AAV vector genome concentrations can vary depending on the protocol, target location on the genome, DNA impurities and the intactness of the genome. Use of a multiplex dPCR approach provides a high-resolution characterization of the viral vector genomes.
Viral titers of AAV reference standards determined using qPCR and an ITR assay may significantly deviate from viral titers obtained using dPCR due to e.g., the standard curve method and/or use of a theoretical, stoichiometric conversion factor to obtain the titer based on the ITR concentration.

ITR secondary structures

The inverted terminal repeats (ITRs) of AAVs include strong secondary structures, sequence repeats and a high GC content, which may limit accessibility of targets during PCR.
Restriction digest with *Hpa*II releases any secondary structure in the DNA that may interfere with access to the target sequence. *Hpa*II enzymes (Thermo Scientific *Hpa*II (10 U/µL, cat. nos. ER0511, ER0512) or Invitrogen Anza 93 *Hpa*II (cat. no. IVGN0936)) have been extensively tested and show high compatibility with the QIAcuity Probe PCR Mix. Restriction digest of ITR secondary structures can directly take place in the PCR mix.
Compatibility of the QIAcuity Probe PCR chemistry with other restriction enzymes should be tested individually before starting with the determination of viral genome titers.

Protocol: AAV particle processing without use of Proteinase K

This protocol is optimized for processing of AAV particles of different purities for vector genome titer determination using a QIAcuity dPCR system. This protocol does not require a Proteinase K digest of AAV capsids.

Important points before starting

- See "Important Notes" on page 11.
- The CGT Viral Vector Lysis Kits have been optimized for processing of AAV particles. Other viral vectors such as Adenovirus particles can be processed following the same procedure.
- AAV particles of different titers can be processed following the same protocol. Dilution series must be adjusted to fit into the dPCR concentration range accordingly. Additional information can be found in the Appendix: Recommendations on dilution steps throughout the processing workflow using the CGT Viral Vector Lysis Kit.
- Long-term storage of AAV samples and lysates is not recommended.
  If a Proteinase K procedure is required, follow "Protocol: AAV particle processing using Proteinase K" on page 18.

Things to do before starting

- Viral vector samples with a high expected titer (e.g., titer > 1×10^{11} vg/mL) may be diluted using the corresponding sample storage buffer supplemented with 0.01% CGT Sample Stabilizer before starting with the processing workflow described below. Alternatively, sample dilutions can be performed in protein low binding tubes.

- 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 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.
- Dilute the CGT Sample Stabilizer stock from 10% (v/v) to a 1% (v/v) working solution using Nuclease Free-Water. 1% working solution can be stored at 2–8°C. Mix thoroughly before use.
- Thaw kit components before starting the particle processing and mix all components (also components stored at RT) right before use. Mix the CGT Lysis Buffer by carefully inverting 3 times before use. The CGT Lysis Buffer tends to intensively foam, hence, make sure to pipette liquid and not foam. However, the foam layer above the liquid does not affect lysis performance.
- 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.

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. The viral particles should be added 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). Afterwards, cool down the reaction at 4°C for 5 min. Spin

* 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.

down and mix properly by pipetting 5 times up and down 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 (e.g., during chromatography procedure), this step should not be skipped. However, in this case, the DNase I enzyme can be replaced by Nuclease-Free Water. Incubation at 37°C is not needed.

Table 1. DNase I reaction setup

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

3. Prepare a viral vector lysis mixture according to Table 2. The sample should be added last. 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.
   Example: The viral particles are 10x serially diluted for four times and only the last dilution step is incubated at 95°C for 10 min.
   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.

Table 2. Viral vector lysis reaction setup

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

4. Dilute the lysates from step 3 according to Table 3. 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 100x taking together step 4 and step 5. A 200x dilution is recommended.

   Example: A viral vector sample is being diluted 20x using the CGT Dilution Buffer in step 4 as described in Table 3. A 10x dilution would then be recommended in step 5 as shown in Table 4. At least a 5x dilution would be needed in step 5. Higher dilutions can be performed without concerns.

Table 3. Lysate dilution setup

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

* Total reaction volume can be scaled up or scaled down to 20 µL. Number of required dilution steps is dependent on the expected viral titer and can be adjusted accordingly.

5. Prepare the PCR reaction mix using the QIAcuity Probe PCR Kit according to Table 4 in a standard PCR plate. Seal the plate and mix thoroughly by vortexing the reaction mix 5 times, 1 s each 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 100x taking together step 4 and step 5. A 200x dilution is recommended.

   Note: It is recommended to prepare a 10% surplus of PCR reaction mix to be able to completely transfer the required volume to the nanoplate.

Table 4. PCR reaction setup

Component	Nanoplate 8.5K (24-well, 96-well)	Nanoplate 26K (24-well)
QIAcuity Probe PCR Master Mix	3 µL	10 µL
CGT dPCR Assay, 20x†	0.6 µL	2 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*
Nuclease-Free Water	7.05 µL	23.5 µL
Total reaction volume	12 µL	40 µL

* Lysate volume is variable depending on required dilution. The water in the PCR reaction can be fully exchanged by viral lysate.

† 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.

‡ 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.

6. Resuspend the PCR mix and transfer the appropriate volume to a nanoplate. Seal nanoplate and load into the QIAcuity instrument. Start run.
   Additional information regarding PCR setup, recommended cycling, and imaging conditions can be found in "Protocol: Absolute quantification of viral vectors using CGT dPCR Assays" on page 23 of this handbook. Steps 1–2 are already covered in this protocol.

Protocol: AAV particle processing using Proteinase K

This protocol is optimized for processing of AAV samples of different purities for vector genome titer determination using a QIAcuity dPCR system. This protocol includes a Proteinase K digest of AAV capsids.

Important points before starting

- Before beginning, read chapter "Important Notes" in this handbook on page 11.
- The CGT Viral Vector Lysis Kits have been optimized for processing of AAV particles. Other viral vectors such as Adenovirus particles can be processed following the same procedure.
- AAV particles of different titers can be processed following the same protocol. Dilution series must be adjusted to fit into the dPCR concentration range accordingly. Additional information can be found in the Appendix: Recommendations on dilution steps throughout the processing workflow using the CGT Viral Vector Lysis Kit.
- Long-term storage of AAV samples and lysates is not recommended.
- If a Proteinase K-free procedure is required, follow "AAV particle processing without the use of Proteinase K" on page 9.

Things to do before starting

- Viral vector samples with a high expected titer (e.g., titer > 1×10^{11} vg/mL) may be diluted using the corresponding sample storage buffer supplemented with 0.01% CGT Sample Stabilizer before starting with the processing workflow described below. Alternatively, sample dilutions can be performed in protein low binding tubes.

- 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 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.
- Dilute the CGT Sample Stabilizer stock from 10% (v/v) to a 1% (v/v) working solution using nuclease free-water. 1% working solution can be stored at 2–8°C. Mix thoroughly before use.
- Thaw kit components before starting the particle processing and mix all components (also components stored at RT) right before use. Mix the CGT Lysis Buffer by carefully inverting 3 times before use. The CGT Lysis Buffer tends to foam intensively, hence, make sure to pipette liquid and not foam. However, the foam layer above the liquid does not affect lysis performance.
- 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.

Procedure

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 5. The viral particles should be added 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). Afterwards, cool down the reaction at

* Kunitz units are commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A260 of 0.001 per minute per mL at 25° C, pH 5.0, with polymerized DNA as the substrate.

4°C for 5 min. 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.

Important: Even if removal of residual DNA contaminants in the sample has already taken place in the upstream sample preparation (e.g., during chromatography procedure), this step should not be skipped. In this case, the DNase I enzyme can be exchanged by Nuclease-Free Water. Incubation at 37°C is not needed.

Table 5. DNase I reaction setup

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

2b. Add 2 µL Proteinase K per 50 µL reaction from step 2a. 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 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 6. The sample should be added last. 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.

   Example: The viral particles are 10x serially diluted for 4 times and only the last dilution

step is incubated at 95°C for 10 min.

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.

Table 6. Viral vector lysis reaction setup

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

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

4. Dilute the lysates from step 3 according to Table 7. 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 100x taking together step 4 and step 5. A 200x dilution is recommended.

   Example: A viral vector sample is being diluted 20x using the CGT Dilution Buffer in step 4 as described in Table 3. A 10x dilution is recommended in step 5 as shown in Table 8. At least a 5x dilution is needed. Higher dilutions can be performed without concerns.

Table 7. Lysate dilution setup

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

* Total reaction volume can be scaled up and scaled down to 20 µL. Number of needed dilution steps is dependent on the expected viral titer and can be adjusted accordingly.

5. Prepare the PCR reaction mix using the QIAcuity Probe PCR Kit according to Table 8. PCR reaction setup in a standard PCR plate. Seal the plate and mix thoroughly by vortexing the reaction mix 5 times, 1 s each 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 100x taking together step 4 and step 5. A 200x dilution is recommended.

   Note: It is recommended to prepare a 10% surplus of PCR reaction mix to be able to completely transfer the required volume to the nanoplate.

Table 8. PCR reaction setup

Component	Nanoplate 8.5K (24-well, 96-well)	Nanoplate 26K (24-well)
QIAcuity Probe PCR Master Mix	3 µL	10 µL
CGT dPCR Assay, 20x†	0.6 µL	2 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*
Nuclease-Free Water	7.05 µL	23.5 µL
Total reaction volume	12 µL	40 µL

* Lysate volume is variable depending on required dilution. The water in the PCR reaction can be fully exchanged by viral lysate.

† Custom designed assays can be used. Start with recommended primer 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

6. Resuspend the PCR mix and transfer the appropriate volume to a nanoplate. Seal nanoplate and load into the QIAcuity instrument. Start run.
   Additional information regarding PCR setup, recommended cycling and imaging conditions can be found in "Protocol: Absolute quantification of viral vectors using CGT dPCR Assays" on page 23 of this handbook. Steps 1 and 2 are already covered in this protocol.

Protocol: Absolute quantification of viral vectors using CGT dPCR Assays

This protocol describes how to setup a QIAcuity PCR for a vector genome titer determination of viral vector lysates generated using a CGT Viral Vector Lysis Kit and CGT dPCR Assays.

Important points before starting

- The 4x QIAcuity Probe PCR Master Mix contains the QuantiNova DNA polymerase, which is inactive at room temperature. The PCR protocol must start with a mandatory initial incubation step of 2 min at 95°C to activate the enzyme.
- A fluorescent dye is provided as a component of the QIAcuity Probe PCR Master Mix, 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.

Things to do before starting

- Resuspend the lyophilized CGT dPCR Assays: Spin down the tube before opening it for the first time. Add 330 µL TE buffer to the tube to obtain a 20x stock and leave at room temperature for 20 min. Vortex and spin down briefly.
- Thaw the QIAcuity Probe PCR Master Mix and mix properly.

Procedure

1. Prepare the PCR reaction mix using the QIAcuity Probe PCR Kit according to Table 9 in a standard PCR plate. Seal plate and mix thoroughly by vortexing the reaction mix 5 times, 1 s each, and incubate for 10 min at room temperature.

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

Table 9. PCR reaction setup

Component	Nanoplate 8.5K (24-well, 96-well)	Nanoplate 26K (24-well)
QIAcuity Probe PCR Master Mix	3 µL	10 µL
CGT dPCR Assay 1, 20x†	0.6 µL	2 µL
Optional: Additional CGT Assays (2, 3, 4, 5) for up to fiveplex reaction‡	0.6 µL	2 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*
Nuclease-Free Water	variable	variable
Total reaction volume	12 µL	40 µL

* Lysate volume is variable depending on required dilution. The water in the PCR reaction can be fully replaced by viral lysate.
† 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 CGT dPCR Assays or gene of interest assays for a multiplex reaction to detect multiple targets at once. Important: Dye combination must be different from those used in the 20x CGT dPCR Assay 1. For dye recommendations and the corresponding channels available on the QIAcuity, see the QIAcuity User Manual (**www.qiagen.com/HB-2717**) or the QIAcuity User Manual Extension: Application Guide (**www.qiagen.com/HB-2839**).
§ 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

2. Resuspend the PCR mix 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 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 10.
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 according to Table 11*.
3. Place the Nanoplate into the QIAcuity instrument and start the dPCR program.

Table 10. Cycling conditions

Step	Time	Temperature
Initial heat activation	2 min	95°C
2-step cycling (40 cycles)		
Denaturation	15 s	95°C
Combined annealing and extension	30 s	60°C

* Always start with recommended cycling conditions. Temperature during annealing/extension and number of cycles might vary when using custom designed assays or assays from other suppliers. 60°C is the optimum for the CGT dPCR assays.

Table 11. Imaging settings*

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

* 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 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 on setting up the plate layout.
2. After the run is completed, the raw data are automatically sent to the QIAcuity Software Suite.
3. For data analysis, open the QIAcuity Software Suite and select the individual Nanoplate for the analysis in **Plate Overview** of the Software Suite.

   Note: See the *QIAcuity User Manual Extension: Application Guide* and *QIAcuity User Manual* for details on how to analyze absolute quantification data.

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** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
DNase I digest	
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 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 flipping. • Suboptimal buffer conditions for efficient DNase digest: High viscosity of the CGT DNase I Buffer. Make sure to pipette indicated volume without air bubbles. • Saturated DNase I digest: Try diluting AAV sample (e.g., 1:10) in protein low binding tubes in the storage buffer of the viral particle sample or preferably in the storage buffer supplemented with 0.01% CGT Sample Stabilizer before setting up the DNase I digest.
Reaction setup	• CGT DNase I Buffer must be used for the DNase I reaction. The buffer composition has been optimized for removal of DNA impurities in AAV samples. Use of alternative buffers may lead to preliminary capsid opening and viral genome loss. • Cool-down at 4°C is required after incubation at 37°C. Deviation from protocol may lead to underquantification of the viral genome titer.
Sample storage after digest	• Underquantification of the viral genome titer: Sample storage after DNase I digest is not recommended. The DNase I enzyme is not inactivated at this step and is still able to digest accessible DNA. Sample freezing and thawing may lead to capsid opening and digest of the viral genome by the active enzyme.
Capsid lysis	

	Comments and suggestions
Capsid opening within nanoplate partitions	• Depending on viral particles of interest capsid lysis efficiency might be reduced within the partitions. Make sure to lyse particle capsids before transfer into nanoplates.
Insufficient capsid lysis	• DNase I step not performed before entering lysis step: DNase I reaction should not be skipped. Buffer composition of particles is essential for optimal lysis. Addition of DNase I enzyme to the reaction can be skipped. Addition of the CGT DNase I buffer into the DNase I reaction is essential. • Incubation at 95°C for 10 minutes skipped: Heat incubation must be performed for efficient capsid lysis and optimal accessibility of genome targets to be amplified during PCR.
Proteinase K	• Proteinase K step skipped: Proteinase K use is optional. Proteinase K-free workflow is recommended. • 95°C incubation for 10 minutes skipped: Heat step must be performed for Proteinase K inactivation. Insufficient inactivation leads to digest of the polymerase in the PCR step. • Insufficient capsid opening: Do not vortex Proteinase K reaction setup.
Reaction setup	• Cool-down at 4°C is required after incubation at 95°C. Deviation from protocol may lead to underquantification of the viral genome titer.
Serial dilution of lysed capsids	
Total dilution lower than 100x	• A 100x dilution (taking together dilution steps after lysis and dilution in the PCR reaction setup) must be at least performed to avoid inhibitory effects. Please ensure proper dilution series and dilute the raw lysate at least 100x or the recommended 200x.
Dilution performed in water	• Dilution in water may 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 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	• Insufficient *Hpa*II restriction: please follow instruction of PCR reaction setup with restriction enzymes as described in the protocol. • *Msp*I enzyme used instead of *Hpa*II in the restriction digest: *Msp*I has low compatibility with the QIAcuity Probe Mix. Please follow instruction described in protocol. • Choice of restriction enzyme: If using other enzymes than those recommended in the protocols, make sure to test compatibility

	Comments and suggestions
	with the QIAcuity Probe PCR chemistry beforehand and optimize ITR secondary structure digest. • Prolonged incubation of the restriction enzymes with the QIAcuity Probe PCR Mix: Freshly prepare the Master Mix and do not incubate for longer than 90 minutes before adding the diluted lysate.
No positive partitions	• Sample input below LOD. • Sample with low titer or too highly diluted: Try reducing dilution steps while always keeping a minimum of 100x dilution after capsid lysis. • 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. • Imaging setup: Check channel choice and match with probe dyes. • Inhibition: Lysates must be diluted at least 100x after lysis (taking together dilution and PCR step). • Prolonged initial denaturation: Ensure to run the PCR using the QIAcuity Probe PCR mix with an initial denaturation of 2 min at 95°C. 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. • Dilution of lysates: Minimum dilution of 100x is a must, 200x is recommended. • 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.

	Comments and suggestions
Titer	
Titer higher than expected	• Insufficient DNase I digest (see troubleshooting chapter "DNase I digest". • Underestimation of titer with reference method (e.g., conversion from qPCR to dPCR; titer provided by supplier of reference standards not accurate).
Titer lower than expected	• Sample handling: Dilution of AAV particles in water may lead to particle damaging and loss of quantifiable genome during downstream processing. • Insufficient capsid opening (see troubleshooting chapter "Capsid lysis"). • Insufficient DNase I inactivation (incubation at 95°C for 10 minutes). • Poor PCR/assay performance (see troubleshooting chapter "PCR").
Inconsistent titer determination	• See troubleshooting chapter "Handling and storage."
Two non-ITR assays lead to different titer	• Sample of interest might contain fragmented genomes or mispackaged genomes. • *Hpa*II digest not properly performed: Secondary structures of ITRs have a greater impact on targets in close vicinity compared to targets with a higher distance. • PCR assays do not perfectly match vector genome (e.g., SNPs in binding sites of primers and/or probes). • Poor assay performance: See troubleshooting chapter "PCR" and design guidelines summarized in the appendix of this handbook.
Discrepancy between titer determined using an ITR and a non-ITR assay	• ITR titer is dependent on multiple factors such as the processing protocol, efficient removal of ITR secondary structures before PCR quantification, buffer conditions and fragmentation and mispackaging state of viral particles of interest. • Theoretically, stoichiometric factor (e.g., factor of 2 between ITR and non-ITR targets) should not be applied for conversion of the ITR target titers to non-ITR target titers.
Handling and storage	
Inconsistent results	• Make sure to mix reagents such as the CGT Lysis Buffer 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 Viral Vector Lysis protocols. When using automatic pipettors, identical pipetting settings should be used by

	Comments and suggestions
	all operators (e.g., pre-dispense mode recommended over direct pipetting mode).
Provided reagents in the kit not sufficient	• CGT Sample Stabilizer: Make sure to dilute the provided stock from 10% (v/v) to 1% (v/v) in water before use. • CGT Lysis Buffer: Provided lysis buffer is sufficient for a total of four 10x dilutions (50 µL reactions). • CGT Dilution Buffer: Provided dilution buffer is sufficient for in total four 10x dilutions (50 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.
Storage	• (Long-term) storage of intermediate workflow products not recommended.
Pipetting volume	• Ensure following lower and higher limit of your pipette. We recommend to not pipette volumes lower than 1 µL. Take viscosity of reagents into consideration and adjust pipetting setup accordingly.

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Appendix: Transfer of qPCR assays to dPCR and design guidance for custom designed assays

The CGT Viral Vector 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 been already 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 give 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-and-thaw cycles.

Transfer of qPCR assays to dPCR

- All rules for proper real-time PCR assay design apply to dPCR
- Care should be taken 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 Probe PCR chemistry

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

Appendix: Recommendations on dilution steps throughout the processing workflow using the CGT Viral Vector Lysis Kit

The CGT Viral Vector Lysis Kit allows processing of viral vector samples of different titers. The kit allows to dilute at different steps throughout the workflow. Some dilutions are mandatory and will negatively affect performance if not followed. Other dilutions are optional to accommodate various samples (purified and unpurified) within a broad range of genome titers.

Required dilutions throughout the processing workflow

- **Step 2: DNase I reaction**
  Viral vector samples can be directly added to the DNase I reaction without initial dilution. Samples are diluted 1:10 in this step. This results in a total dilution of 10x.
- **Step 3: Lysis of capsids**
  After DNase I digest, samples are diluted 1:10 in the CGT Lysis Buffer. This results in a total dilution of 100x taking together step 2 and step 3.
- **Step 4: Dilution series**
  Option 1: It is recommended to dilute the lysates 1:20 with CGT Dilution Buffer. This would result in a total dilution of 2,000x taking together step 2, step 3, and step 4.
  Option 2: The lysates can be diluted 1:10 with CGT Dilution Buffer. This would result in a total dilution of 1,000x taking together step 2, step 3, and step 4.
  Option 3: The lysates can be diluted in a dilution series. The CGT Viral Vector Lysis Kit provides CGT Dilution Buffer sufficient for four 50 µL 1:10 dilutions. This would result in a total dilution of 1,000x (first dilution in dilution series) to 1,000,000x (last dilution in dilution series) when taking together step 2, step 3, and step 4.
- **Step 5: dPCR setup**
  Option 1 from step 4: It is recommended to dilute the samples 1:10 in the PCR reaction.

For that, 1.2 µL diluted lysate is used for a 12 µL PCR reaction. This would result in a total dilution of 20,000x when taking together step 2 – step 5.

Alternatively, a 1:5 dilution is sufficient without negatively impacting PCR performance. For that, 2.4 µL diluted lysate is used for a 12 µL PCR reaction. This would result in a total dilution of 10,000x when taking together step 2 – step 5*.

Option 2 from step 4: It is recommended to dilute the samples 1:20 in the PCR reaction. For that, 0.6 µL diluted lysate is used for a 12 µL PCR reaction. This would result in a total dilution of 20,000x when taking together step 2 – step 5.

Alternatively, a 1:10 dilution is sufficient without negatively impacting PCR performance. For that, 1.2 µL diluted lysate is used for a 12 µL PCR reaction. This would result in a total dilution of 10,000x when taking together step 2 – step 5*.

Option 3 from step 4: The sample from the first dilution step requires at least a 1:10 dilution in the PCR reaction. For that, 1.2 µL diluted lysate is used for a 12 µL PCR reaction. This would result in a total dilution of 10,000x when taking together step 2-step 5*. All other samples from the dilution series are sufficiently diluted. The input volume into the dPCR reaction can be chosen freely.

* Keeping the dPCR detection range (2.5 cop/µL – 15,000 cop/µL on an 8.5K nanoplate), higher dilutions can be chosen without concerns.

Table 12. Overview total dilutions

Step	Dilution within step	Total dilution
Step 2: DNase I reaction	1:10	10x
Step 3: Capsid lysis	1:10	100x
Step 4: Dilution series Option 1 Option 2 Option 3	 1:20 1:10 1:10	 2,000x 1,000x 1,000x – 1,000,000x
Step 5: dPCR setup Option 1 from step 4 Option 2 from step 4 Option 3 from step 4: First sample from dilution series Option 3 from step 4: All other samples from dilution series	 1:5 – 1:10 1:10 – 1:20 1:10 variable	 10,000x – 20,000x 10,000x – 20,000x 10,000x >10,000x – 1,000,000x

Initial dilution of viral vector samples with an expected titer of > 1 x 10^{11} vg/mL

- Viral vector sample with an expected high titer may be diluted using the corresponding sample storage buffer supplemented with 0.01% CGT Sample Stabilizer before starting with the processing workflow. Addition of the CGT Sample Stabilizer to the storage buffer might reduce the number of samples that can be processed using the CGT Viral Vector Lysis Kit.
  Alternatively, sample dilutions can be performed in protein low binding tubes.

Initial dilution of viral vector samples with an expected genome titer of < 1 x 10^{11} vg/mL

- Initial sample dilution is not recommended. Additional dilutions, if needed, can be performed during capsid lysis and the downstream dilution steps. The CGT Viral Vector Lysis Kit has enough lysis and dilution buffers for 4 1:10 dilutions each in a final reaction volume of 50 µL.

Ordering Information

Product	Contents	Cat. no.
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
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
dPCR CGT Assay ITR2/5 (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250230
dPCR CGT Assay ITR2/5 (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250231
dPCR CGT Assay ITR2/5 (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250232
dPCR CGT Assay bGH polyA (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250233
dPCR CGT Assay bGH polyA (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction)	250234

	Premixed primers and probe, lyophilized.	
dPCR CGT Assay bGH polyA (Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250235
dPCR CGT Assay GFP (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250236
dPCR CGT Assay GFP (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250237
dPCR CGT Assay GFP (Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250238
dPCR CGT Assay WPRE (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250239
dPCR CGT Assay WPRE (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250240
dPCR CGT Assay WPRE (Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250241

dPCR CGT Assay SV40 promoter (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250242
dPCR CGT Assay SV40 promoter (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250243
dPCR CGT Assay SV40 promoter (Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250244
dPCR CGT Assay SV40 polyA (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250245
dPCR CGT Assay SV40 polyA (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250246
dPCR CGT Assay CMV promoter (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250247
dPCR CGT Assay CMV promoter (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250248
dPCR CGT Assay CMV promoter (Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction)	250249

	Premixed primers and probe, lyophilized.	
dPCR CGT Assay hGH polyA (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250250
dPCR CGT Assay hGH polyA (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250251
dPCR CGT Assay CMV enhancer (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250252
dPCR CGT Assay CMV enhancer (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250253
dPCR CGT Assay CMV enhancer (Cy5) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250254
dPCR CGT Assay AMP resistance (FAM) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250255
dPCR CGT Assay AMP resistance (HEX) (20x)	For 500 dPCR reactions (8.5K nanoplate, 12 µL per reaction) Premixed primers and probe, lyophilized.	250256

Proteinase K (1 mL, 5 mL)	Proteinase K solution with a concentration of ≥ 20 mg/mL and an activity of ≥ 800 U/mL	RP107B-1 RP107B-5
QIAcuity Probe PCR Kit (1 mL, 5 mL)	Ready-to-use 4x concentrated Master Mix, water	250101 250102
QIAcuity Nanoplate 8.5K 96-well (10)	10 QIAcuity Nanoplates 8.5K with 96 wells 11 Nanoplate Seals	250021
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,	911040

	training, and 1 preventive maintenance visit.1 year warranty on labor, travel, and parts.	
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 HEK293 resDNA Quant Kit (96)	For 96 reactions: QIAcuity HEK293 resDNA Quant Master Mix (4x) lyophilized, Positive Control, Internal Control, RNAse-Free Water	250224
HEK293 resDNA Quant Standard Kit	HEK293 resDNA Quant Standard, Rehydration Buffer	250225
QIAcuity CHO resDNA Quant Kit (96)	For 96 reactions: QIAcuity CHO resDNA Quant Master Mix, Positive Control, Internal Control, RNase-Free Water	250222
CHO resDNA Quant Standard Kit	CHO resDNA Quant Standard, Rehydration Buffer	250223
QIAcuity *E. coli* resDNA Quant Kit (96)	For 96 reactions: QIAcuity *E. coli* resDNA Quant Master Mix, Positive	250220

	Control, Internal Control, RNase-Free Water	
E. coli resDNA Quant Standard Kit	*E. coli* resDNA Quant Standard, Rehydration Buffer	250221

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Document Revision History

Revision	Description
03/2023	Initial release

Notes

Limited License Agreement for CGT Viral Vector Lysis Kits and CGT dPCR Assays

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