# Determination of adeno-associated virus (AAV) titers using the QIAcuity® Digital PCR System

Domenica Martorana[1], Anne Schieren[1], Jonathan Thorn[1], Mario Quinto[1], Alex Deiulio[2], Andrzej Noyszewski[2], Pavle Matak[3], Daniel Lofgren[4]

[1] QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany; [2] Agathos Biologics, 4837 Amber Valley Pkwy, 58104 Fargo, ND, USA

[3] QIAGEN Sciences LLC, 19300 Germantown Rd, 20874 Germantown, MD, USA; [4] QIAGEN AB, Torshamnsgatan 35 SE-16440 Kista, Sweden

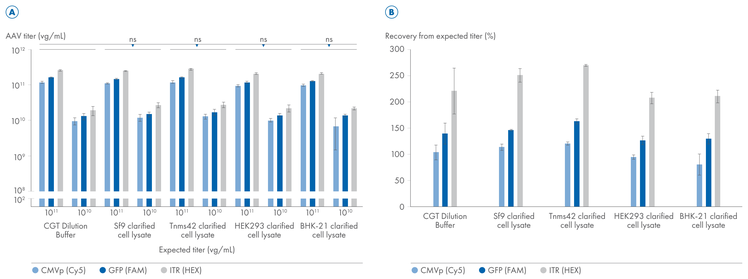
## Novel CGT viral vector lysis kit enables a standardized workflow from cell lysate to absolute and precise quantification of viral titers

Cell and gene therapies seek to target previously untreatable diseases at their source using individualized treatments. However, developing safe and effective cell and gene therapies requires strict monitoring at all stages of the development process. Adeno-associated virus (AAV) has turned into a primary modality for efficient gene therapy applications. The process of generation and purification of the viral vectors require precise quality control to enable safe and reliable dosing during clinical studies or patient care. The ability to accurately and reproducibly quantify vector titers is essential for safe and effective AAV-based gene therapies. The AAV genome is a key component for vector quantification. While several quantification assays are available, here, we outline a standardized processing and quantification workflow compatible with many AAV preparations of different purities. We show that an optimized capsid lysis procedure together with an increased target accessibility allows for a robust genome titration throughout the whole viral vector manufacturing process using PCR.

qPCR is a widely used method for AAV quantification due to its sensitivity and ease of use. Nevertheless, well-characterized DNA standards and assays are needed for accurate quantification. Digital PCR enables absolute quantification with unprecedented precision and a higher tolerance towards inhibitors without the need for any standards. Additionally, more than one region of interest can be quantified at once leading to further information on genome intactness.

## Highly accurate and reproducible AAV viral titer determination in different background matrices

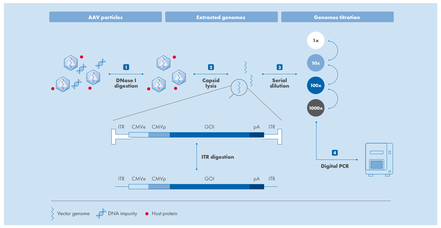
The Cell and Gene Therapy (CGT) Viral Vector Lysis Kit is compatible with AAV samples of different purities and allows reliable AAV titer determination on a QIAcuity dPCR System. The clarified cell lysates from Sf9 (insect cells), Tnms42 (insect cells), HEK293 and BHK-21 did not significantly affect the viral vector titer quantification.




**Robust AAV titer determination. A** A purified AAV2 reference standard ($10^{11}$ vg/mL and $10^{10}$ vg/mL; Vigene) was spiked into CGT Dilution Buffer as well as clarified cell lysates derived from Sf9 (insect cells), Tnms42 (insect cells), HEK293 and BHK-21. The particles were processed using the CGT Viral Vector Lysis Kit. After capsid lysis, the samples were serially diluted (10x, 5x, 4x, 4x). The last three dilution steps were used for titer determination. Quantification was performed in technical duplicates using 8.5k nanoplates and the CGT dPCR CMV promoter, GFP and ITR assays in the FAM, HEX and Cy5 channels of the QIAcuity dPCR System. The expected AAV titer is based on titer information provided by the supplier. A paired student's t-test was performed to determine significance (ns not significant *, p<0.05 **, p<0.01***, p≤0.001). **B** The percentage of measured titer from the expected titer was calculated using all data points from $10^{11}$ vg/mL and $10^{10}$ vg/mL AAV spike-in samples.

## AAV vector titer determination following the CGT viral vector workflow

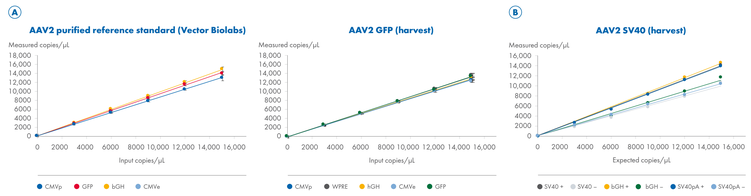
AAV particles of different serotypes and different purity grades are efficiently processed in four standardized steps using the Cell and Gene Therapy Viral Vector Lysis Kit ready for genome titration on a QIAcuity dPCR System.




**AAV sample processing and titer determination using the CGT Viral Vector Lysis Kit.** In a first step, AAV samples are treated with DNaseI to remove DNA impurities. In step 2, DNaseI-treated samples are lysed with or without Proteinase K. In step 3, the lysates are serially diluted, and the viral titers determined on a QIAcuity dPCR instrument in step 4.

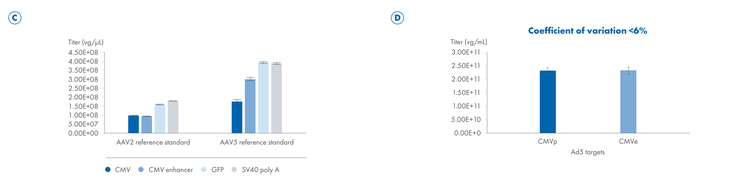
## Absolute titer quantification of viral vectors using the QIAcuity dPCR System

The CGT Viral Vector Lysis Kit reliably processes viral vectors of different purities. Quantification with high accuracy over a broad dynamic range can be achieved using the CGT dPCR assays. Quantification of multiple targets on the genome increases insights on sample quality.




| AAV2 | CMVe | CMVp | WPRE | hGH pA | GFP |
|---|---|---|---|---|---|
| **Coefficient of variation (8.5k) [%]** | 2.4 | 2.7 | 2.7 | 3.6 | 3.4 |
| **Coefficient of variation (26k) [%]** | 2.9 | 2.4 | 2.1 | 1.7 | 3.7 |
| **$R^2$** | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |

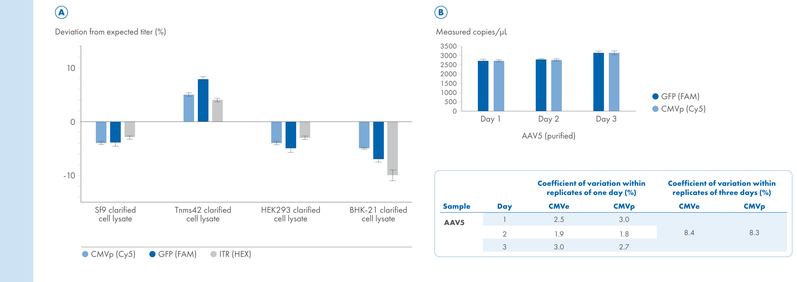
**ITR digestion improves the quantification of ITR and non-ITR targets. A** AAV2 samples were processed using the CGT Viral Vector Lysis Kit and quantified on a QIAcuity dPCR System using 8.5k nanoplates and CGT dPCR assays. The CGT dPCR assays were run in triplex reactions in the FAM™, HEX™ and Cy®5 channels. The samples were serially diluted in 6 steps from 15,000 cop/µL down to 2.5 cop/µL. **B** Coefficients of variation, $R^2$ values and deviations between the quantification on 8.5k and 26k nanoplates are shown.




**Titration of AAV and Ad5 samples. C** DNA was extracted from two purified AAV2 and AAV5 reference standards (Vigene). CGT dPCR Assays targeting the CMV promoter, CMV enhancer, GFP and SV40 polyA regions were used in the dPCR reaction. **D** A purified Adenovirus 5 (Ad5) sample was processed using the CGT Viral Vector Lysis Kit. CGT dPCR Assays targeting the CMV promoter and the CMV enhancer regions were used in a duplex reaction in the HEX and Cy5 channels. 9 technical replicates were run for each assay in an 8.5k nanoplate.

## Robust AAV5 processing and quantification in different background matrices and high repeatability

The genome titer of AAV5 particles in different background matrices can be determined with high accuracy and linearity using the CGT workflow solutions and the QIAcuity dPCR System. The largely standardized workflow enables reliable titration with high inter- and intra-assay precision (less than +/–10%) independent of assays, operators and laboratories.




| Sample | Day | Coefficient of variation within replicates of one day (%) CMVe | CMVp | Coefficient of variation within replicates of three days (%) CMVe | CMVp |
|---|---|---|---|---|---|
| **AAV5** | 1 | 2.5 | 3.0 | | |
| | 2 | 1.9 | 1.8 | 8.4 | 8.3 |
| | 3 | 3.0 | 2.7 | | |

**Reproducible AAV titer determination independent of background matrices and operators. A** An AAV5 reference standard (Vector Biolabs) was processed using the CGT Viral Vector Lysis Kit and quantified using the CGT dPCR assays targeting the CMV promoter, GFP and ITR regions on a QIAcuity dPCR System. The determined vector genome titers for every target were set as expected titer. AAV5 particles were spiked into clarified cell lysates derived from Sf9 (insect cells), Tnms42 (insect cells), HEK293 and BHK-21. The particles were processed using the CGT Viral Vector Lysis Kit. After capsid lysis, the samples were serially diluted (10x, 5x, 4x, 4x). The last three dilution steps were used for titer determination. Quantification was performed in technical duplicates using 8.5k nanoplates and the CGT dPCR CMV promoter, GFP and ITR assays in the FAM, HEX and Cy5 channels of the QIAcuity dPCR System. The deviation from the measured titers to the expected titers was calculated for each target separately. **B** An AAV5 reference standard was processed over 3 days by one operator. Mean quantification of 15 replicates and standard deviation is shown. Coefficient of variations (CV) within the replicates of one day as well as between the replicates of 3 or 4 days are shown.

## Conclusions

- Viral vectors such as AAVs and Ad5 can be reliably processed using the CGT Viral Vector Lysis Kit. Genome titers can be accurately determined using dPCR and the CGT dPCR assays without the need for references or standard curves.
- Clarified cell lysates derived from different cell lines as matrix background do not affect sample processing and downstream quantification.
- High repeatability in titer determination between different operators, days and laboratories.
- Viral genome titer can be determined in singleplex and multiplex reactions to gain a more complete picture on the genome state.
- Broad dynamic range allows titration of samples with high and low titers.



Sample to Insight