

Replication Competent Virus Quantitation Kits (qPCR Method)

Replication competent lentivirus/retrovirus (RCL/RCR) can integrate into the host cell genome and may cause long-term effects, for example, the risk of insertional mutagenesis, potentially leading to cancer. Co-infection of a cell with both rcAAV and a therapeutic AAV vector may affect the conversion of single to double-stranded genomes in the presence of adenovirus, and may also lead to mobilization of the vector upon adenovirus infection.

Product Introduction

SHENTEK® viral DNA and RNA extraction kit ensures efficient and reproducible purification of DNA and RNA viruses from various samples, such as cell substrate or culture up to at 10⁷ cells/mL, virus harvests, bulk products or final products. This kit supports both manual sample preparation and automated extraction using the rHCDpurify® instrument, with customized extraction protocols available for integration with other automated extraction platforms to accommodate specific sample types and workflow requirements.

SHENTEK® RCL/RCR quantitation kits and rcAAV quantitation kit utilize real-time PCR technology to detect the replication competent virus (RCVs) in cell lysate following multiple rounds of infection and cell-based amplification, as well as to directly quantify the replication competent virus concentration in the cell-free supernatant or the extracted samples from cell substrates. The assays demonstrate high specificity and robust performance and have been validated comprehensively in compliance with applicable pharmacopoeia requirements.



SHENTEK® Viral Nucleic Acid Extraction Kit



SHENTEK® Replication Competent Virus Quantitation Kits

Product Number	Product Name	Quantity
1506730	SHENTEK® Virus DNA & RNA Extraction Kit	50 Reactions
1403441	SHENTEK® Replication-Competent Lentivirus (RCL) Quantitation Kit	100 Reactions
1403442	SHENTEK® Replication-Competent Retrovirus (RCR) Quantitation Kit	100 Reactions
1403444	SHENTEK® rcAAV-2/N Quantitation Kit	100 Reactions*2
1403445	SHENTEK® rcAAV-5/N Quantitation Kit	100 Reactions*2



Regulatory Testing Requirements

Authorities	Regulations	Requirements
USP	<1047> GENE THERAPY PRODUCTS	Cell banks, viral vector production lots, and any resulting ex vivo product lots
EP	5.14. Gene transfer medicinal products for human use	Substrate, vector seed lot, purified bulk or Final lot
ChP	ChP 2025, Volume III, General Principle of Gene Therapy Products for Human Use	Virus seed lots, final lots
FDA	Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) Guidance for Industry. Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up Guidance for Industry.	MCB, vector harvest material, viral vector bank, Ex vivo transduced cells, etc.
EMA	Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells	Starting materials, vector release, final products



Replication Competent Virus Quantitation Assay Validation

Parameter	Acceptance Criteria
Linear range	RCL: 60-6×10 ⁶ copies/μL; RCR: 50-5×10 ⁶ copies/μL; rcAAV: 20-2×10 ⁷ copies/μL
Accuracy	The intra-assay values met the following acceptance criteria: The CV and RE at highest & lowest concentration levels were less than 20% and less than 15% at the intermediate concentration levels;
Quantitation Limit	RCL: 120 copies/reaction (6 copies/μL) RCR: 100 copies/reaction (5 copies/μL) rcAAV-2/N & rcAAV-5/N: 400 copies/reaction (20 copies/μL) CV ≤ 20%, RE ≤ 20%
Detection Limit	RCL: 20 copies/reaction (1.0 copies/μL) RCR: 20 copies/reaction (1.0 copies/μL) rcAAV-2/N: 40 copies/reaction (2 copies/μL) rcAAV-5/N: 80 copies/reaction (4 copies/μL)
Specificity	No cross-reactivity with different production cell substrates, engineered bacteria and fungi
Precision (Repeatability)	The CV of 10 intra-assay values at both high and low concentration levels were less than 15%.
Robustness	Instrument suitability: Good consistent assay performance on different qPCR instruments (e.g. SHENTEK-96S, ThermoFisher ABI 7500, Bio-Rad CFX-96, Roche LightCycler480 II, etc.) Freeze-thaw stability: Stable assay performance for at least 5 freeze-thaw cycles



Characteristics of Virus DNA & RNA Extraction Kit

Parameter	Performance
Application Scope	• Applicable to viral extraction in cell substrate of 10^7 cell/mL or below, and cell-free supernatants. • For cell substrate samples, the fulfillment of the following conditions depend on whether the viruses are integrated into the host genome: ① For the integrated virus genome: The genomes of host cell and culture should be fully extracted for detection, such as RCL/RCL assay. ② For the unintegrated virus genome: Only the supernatant is required for extraction after cell lysis, such as rAAV detection.
Extraction Efficiency	Case 1 - RCL extraction recovery: ① Cell-free supernatant: The average RCL extraction recovery rate is 98.4%. ② Cell matrix sample: The average RCL extraction recovery is 96.4%. Case 2 - rAAV & rcAAV extraction recovery: ① Cell-free supernatant: the average rAAV extraction recovery rate is 95.2%, and the average rcAAV extraction recovery rate is 95.5%. ② Cell matrix sample: The average rcAAV extraction recovery rate is 73.6%.

Standard Curves for SHENTEK® RCL & RCR Quantitation Assay

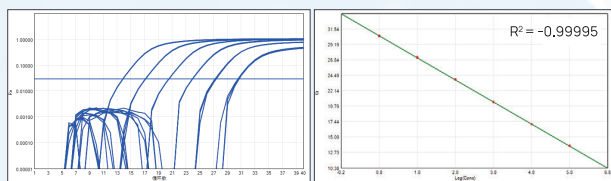


Fig 1. RCL Amplification Curve & Standard Curve

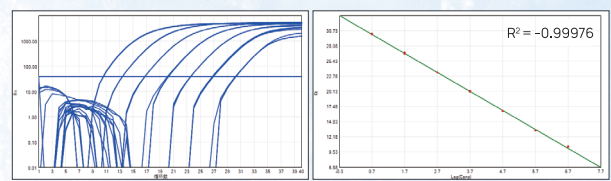


Fig 2. RCR Amplification Curve & Standard Curve

Standard Curves for SHENTEK® rcAAV Quantitation Assay

SHENTEK® rcAAV quantitation kits are designed to evaluate rcAAV contamination levels in two serotypes of rAAV-2/N and rAAV-5/N (N represents any capsid variant within the respective serotype group). The assays are sensitive, accurate and reliable across a broad range of samples, such as rAAV bulk, final products, and total cellular DNA extraction after cell-based amplification. The kits provide simultaneous rapid and specific quantitation of rAAV vector and rcAAV impurity levels. Standard curves for the reference and target genes are generated using corresponding quantitation standards through serial dilution. Importantly, the target gene sequence spans the ITR-rep gene junction, a requisite feature for AAV replication in vivo in the presence of a helper virus.

Before using the rcAAV quantitation kit, please confirm:

- ☑ AAV serotype: rcAAV-2/N and rcAAV-5/N available ('N' represents any capsid variant within the corresponding serotype group.)
- ☑ ITR sequence: The ITR sequence of the AAV vector must match the ITR sequence targeted by the corresponding kit.

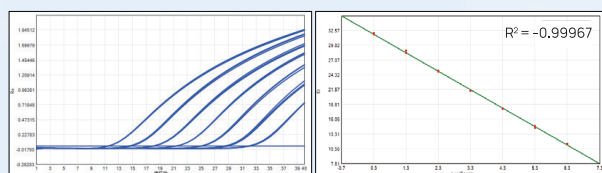


Fig 3. rcAAV-5/N Target Gene Amplification Curve & Standard Curve

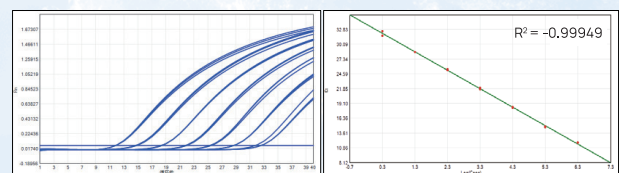


Fig 4. rcAAV-2/N Amplification Curve & Standard Curve

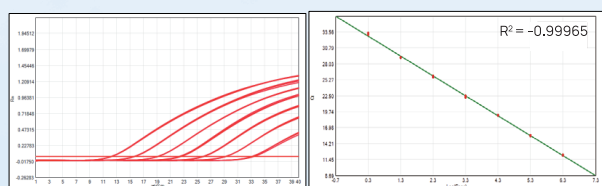


Fig 5. rcAAV-5/N Reference Gene Amplification Curve & Standard Curve

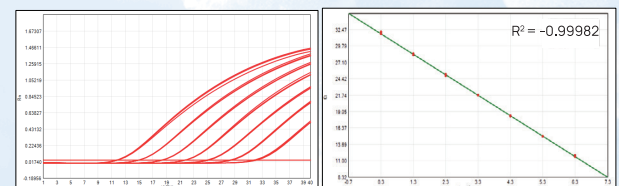


Fig 6. rcAAV-2/N Reference Gene Amplification Curve & Standard Curve



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