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SHENTEK

Replication Competent Virus Quantitation Kits (qPCR Method)

Replication competent lentivirus/retrovirus (RCL/RCR) are capable of integrating in the genome and may confer long-term effects, for example, the risk of insertional mutagenesis, potentially leading to cancer. Confection 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 of 10^7 cells/mL or below,virus harvest, bulk or final products. It is compatible with manual sample preparation, or automated extraction using the rHCDpurify® instrument.

SHENTEK® RCL/RCR quantitation kits and rcAAV quantitation kit use real-time PCR technology to detect the replication competent virus in cell lysate after multiple rounds of infection and cell-based amplification, as well as to directly determine the replication competent virus concentration in the cell-free supernatant or the extracted samples from cell substrates.The assay performance is highly specific and has been validated comprehensively in compliance with 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
NMPA	①Technical Guideline for Pharmaceutical Research and Evaluation of In Vitro Gene Modification Systems (Interim), 2022 Edition ②Technical Guideline for Pharmaceutical Research and Evaluation of Cell Therapy Products (Interim), 2022 Edition ③Technical Guideline for Pharmaceutical Research and Evaluation of Gene Transduction and Modification Systems, 2020 Edition ④Considerations for Quality Control Testing Studies and Non-Clinical Studies of CAR-T Cell Therapy Products, 2018 Edition	Cell banks, Harvest, EOPC, purified bulk, final lots, etc.
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
ChP	ChP 2020, Volume III, General Principle of Gene Therapy Products for Human Use	Virus seed lots, final lots

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/RCR assay. ② For the unintegrated virus genome: Only the supernatant is required for extraction after cell lysis, such as rcAAV 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

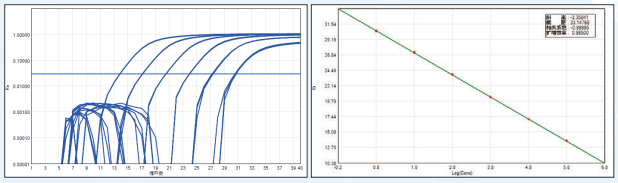

Fig 1. RCL Amplification Curve & Standard Curve

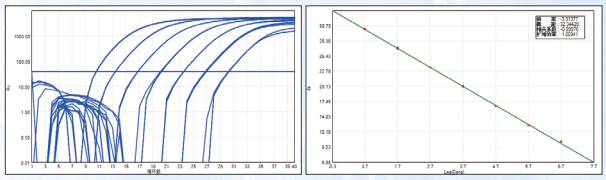

Fig 2. RCR Amplification Curve & Standard Curve

Standard Curves for SHENTEK® rcAAV Quantitation Assay

SHENTEK® rcAAV quantitation kits are used to evaluate rcAAV contamination levels in two serotypes of rAAV-2/N and rAAV-5/N (N means any capsid structures of each serotype). 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 kit provides simultaneous rapid and specific quantitation of rAAV vector and rcAAV impurity levels. Serial dilutions for both standard curves are prepared using the quantitation standard for the measurement of the reference gene and target gene. In addition, 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:

- The serotype of AAV (Currently rcAAV-2/N and rcAAV-5/N quantitation kits available, N means any capsid structures of each serotype);
- The rAAV terminal repeat (ITR) of the vector sample to be tested matches the sequence required by the corrsponding kit.

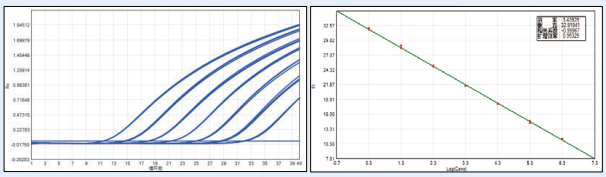

Fig 3. rcAAV-5/N Target Gene Amplification Curve & Standard Curve

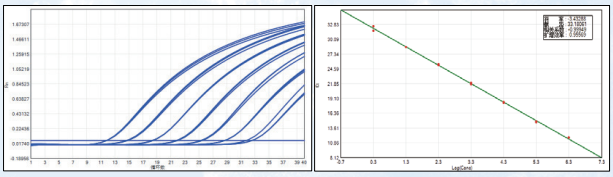

Fig 4. rcAAV-2/N Amplification Curve & Standard Curve

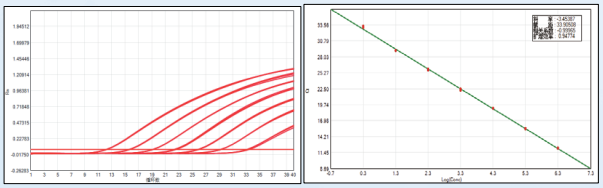

Fig 5. rcAAV-5/N Reference Gene Amplification Curve & Standard Curve

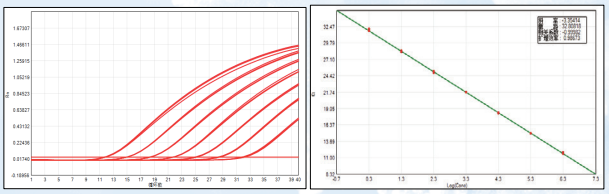

Fig 6. rcAAV-2/N Reference Gene Amplification Curve & Standard Curve

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