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SHENTEK

SHENTEK® Residual Host Cell DNA & RNA Quantitation

Product introduction

The SHENTEK® host cell residual DNA & RNA quantitation systems offer integrated solutions from sample preparation to qPCR assay for specific, rapid and reliable quantitation of residual host cell DNA (rHCD) and RNA (rHCR), covering production cell expression systems, such as bacteria, yeast, insects, mammalian, sensitive quantitation to the fg DNA/μL level. The assays are compatible with SHENTEK® residual host cell DNA sample preparation kit, ensuring highly efficient DNA recovery.The system provides an easy workflow to deliver results in five hours. Internal Positive Control (IPC, VIC assay) is provided for optional use (please consult technical support for use if needed) in every qPCR assay to exclude false negative results. The assay performance has been fully validated and complies with regulatory requirements. These kits have been applied successfully to QC tests for regulatory filings in China, USA, and other countries.

In addition, SHENTEK provides an integrated solution of development and validation of customized kits for specific species/sequence residual DNA/RNA quantitation, as well as optimization of sample pretreatment in biological samples. Customized assay aims to satisfy the detection needs due to different hosts or targets, and meet regulatory filing requirements and can be used for batch release of various biologics.

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SHENTEK® Residual Host Cell DNA Sample Preparation Kits

1.Residual DNA sample preparation kits

Manual and automated sample preparations are used for residual DNA recovery in a broad range of biological products, at different stages of manufacturing process, such as in-process samples, bulk harvest, and final product samples. The rHCDpurify® Sample Preparation System, together with SHENTEK® Sample Preparation Kits offer stable and efficient automated extraction of trace host cell DNA (loading capacity of magnetic particle, of the automated extraction is 50% higher than that of the manual extraction). Efficient and reproducible recovery was validated in varying complex sample mixtures (high protein, high salt, low pH, etc.).

2.Residual RNA sample preparation kit

This kit is used for extraction of residual host cell RNA in a broad range of biological products, and is compatible with the rHCD-purify® Sample Purification System for automated sample preparation. The efficient and reproducible recovery have been validated in different sample mixtures.

Product Number	Product Name	Quantity
SK030203D100	SHENTEK® Residual Host Cell DNA Sample Preparation Kit (for manual extraction)	100 Extracts
1104191	SHENTEK® Residual Host Cell DNA Sample Preparation Kit (for automated extraction)	100 Extracts
1201205	SHENTEK® Residual Host Cell RNA Sample Preparation Kit	100 Extracts

SHENTEK® Residual Host Cell RNA Quantitation Kits

SHENTEK® Residual Host Cell RNA quantitation system, based on real-time PCR technology, is designed for quantitation of residual host cell RNAs in cell and gene therapy products, including various E. coli cell lines that are used for plasmid production, and HEK293T cells used for recombinant vector generation. The kits are prepared with specific pre-designed primers and probes, enabling one-step RNA reverse transcription and amplification,as well as ensuring high efficiency, specificity and sensitivity. SHENTEK® Residual Host Cell RNA Quantitation Kits have been fully validated on different qPCR instruments, in conjunction with SHENTEK® Residual Host Cell RNA Sample Preparation Kit for RNA extracton.

Product Number	Product Name	Quantity
1201201-1	SHENTEK® Residual E. coli RNA Quantitation Kit (2G)	100 Reactions
1201202	SHENTEK® Residual 293T RNA Quantitation Kit	100 Reactions
1201203	RT-PCR Grade Water	16 tubes, 1.5 mL/tube

Validation of Residual Host Cell RNA Quantitation Assays

The qPCR assay performance validation was carried out with reference to USP (Chapter 1225), EP (Chapter 2.6.21), ChP (Chapter 9101) and ICH guideline Q2.

Parameters		Acceptance Criteria
Linear Range		Correlation coefficient: $R^2 > 0.990$; Efficiency: $90\% \le E \le 110\%$;The %CV should be within 15% of nominal concentration, 20% of nominal concentration at LLOQ and ULOQ.
Accuracy		The intra-assay values met the following acceptance criteria:%CV: ≤30%; Recovery: 50% – 150%.
Precision	Repeatability	The values of 10 technical replicates of the same sample meets CV≤15%.
	Intermediate precision	The %CV of 9 inter-assay values at each concentration level (total 3 concentrations) were less than 15% at nominal concentration levels, and 20% at LOQ level.
LOQ		The %CV of all replicates at the LOQ level are less than 20%.
Specificity		No cross-reactivity with nontarget DNA or RNA of the commonly used production cells, engineered bacteria and fungi, plasmid DNA, etc.
Robustness	Freeze-thaw stability	Stable assay performance for at least 5 freeze-thaw cycles.
	Instrument suitability	Good consistency of the assay performance on different qPCR instruments (e.g. HZSKBio® SHENTEK-96S, ThermoFisher ABI 7500, Bio-Rad CFX-96, Bioer FQD-96A, etc.)

SHENTEK® Residual Host Cell DNA Quantitation Kits

Hot-Start DNA Polymerase and PCR buffer solution for the assay are optimized with increased resistance to various reaction inhibitors or interferences. Furthermore, the addition of dUTP/UNG to reaction mix eliminates carry-over contamination and the generation of false positives, with the lowest end of quantitation at fg/μL DNA level. The assay performance has been fully validated, including linear range, accuracy, precision, LOD, specificity and robustness, etc. Full validation reports are available, and meet requirements of pharmacopeial regulations. Internal Positive Control (IPC, VIC assay) is provided for optional use (please consult technical support for use if needed). The development and production of the kits comply with ISO13485 quality standards. These kits have been applied successfully to the QC tests for regulatory filings in US, China and other countries.

Product Number	Product Name	Quantity
1101107-1	SHENTEK® Residual E. coli DNA Quantitation Kit (2G)	100 Reactions
SK030205P100	SHENTEK® Residual Pichia pastoris DNA Quantitation Kit	100 Reactions
SK030222HA100	SHENTEK® Residual Hansenula polymorpha DNA Quantitation Kit	100 Reactions
1101103	SHENTEK® Residual Saccharomyces cerevisiae DNA Quantitation Kit	100 Reactions
1101100-1	SHENTEK® Residual CHO DNA Quantitation Kit (2G)	100 Reactions
SK030208N100	SHENTEK® Residual NS0 & SP2/0 DNA Quantitation Kit	100 Reactions
SK030204V100	SHENTEK® Residual Vero DNA Quantitation Kit	100 Reactions
SK030227V100	SHENTEK® Residual Vero DNA-154 Quantitation Kit	100 Reactions
1101105	SHENTEK® Residual CV-1 DNA Quantitation Kit	100 Reactions
SK030209M100	SHENTEK® Residual MDCK DNA Quantitation Kit	100 Reactions
1101101	SHENTEK® Residual Sf9 & AcNPV DNA Quantitation Kit	100 Reactions
1101102	SHENTEK® Residual Hi5 & AcNPV DNA Quantitation Kit	100 Reactions
1101108	SHENTEK® Residual Human DNA Quantitation Kit (2G)	100 Reactions
1101104-1	SHENTEK® Residual HEK293 DNA Quantitation Kit (3G)	100 Reactions
1101116	SHENTEK® Residual BHK DNA Quantitation Kit	100 Reactions
1403443	SHENTEK® Residual SV40 LTA & E1A DNA Quantitation Kit (2G)	100 Reactions
1101109	SHENTEK® E1A Residual DNA Quantitation Kit	100 Reactions
1101110	SHENTEK® E1B Residual DNA Quantitation Kit	100 Reactions
1101111-1	SHENTEK® Residual Plasmid DNA Quantitation Kit (3G)	100 Reactions
1101123	SHENTEK® Residual PG13 DNA Quantitation Kit	100 Reactions
1101124	SHENTEK® Residual MRC-5 DNA Quantitation Kit	100 Reactions
1101125	SHENTEK® Residual RDF21 DNA Quantitation Kit	100 Reactions

Validation of Residual Host Cell DNA Quantitation Assays

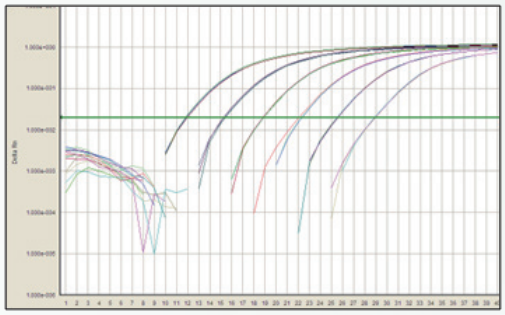
The qPCR assay performance validation was carried out with reference to USP (Chapter 1225), EP (Chapter 2.6.21), ChP (Chapter 9101) and ICH guideline Q2.

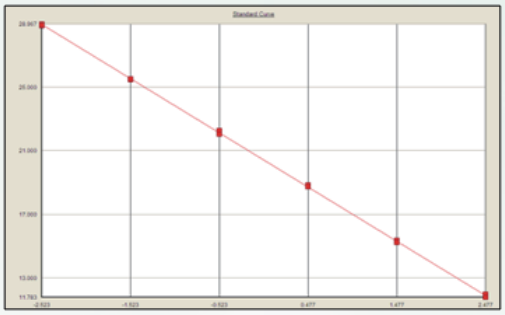
Parameters		Acceptance Criteria
Linear Range		3 or 30 fg/μL to 300 pg/μL (Please refer to the specific User Guide); Correlation coefficient: $R^2 \geq 0.990$; %CV (coefficient of variation) and %RE (relative error): <30%.
Accuracy		DNA recovery using SHENTEK® residual host cell DNA sample preparation kit met the following acceptance criteria:%CV: <20%; Recovery: 70-130%.
Precision	Repeatability	The values of 10 replicates of the same sample meets CV≤15%.
	Intermediate precision	The %CV of 9 inter-assay values at each concentration level (total 3 concentrations) were less than 15% at nominal concentration levels, and 20% at LOQ level.
LOQ		The %CV of all replicates at the LOQ level are less than 20%.
Specificity		No cross-reactivity with nontarget DNA of the commonly used production cells, engineered bacteria and fungi, plasmid DNA, etc.
Robustness	Reference DNA robustness	DNA fragmentation by sonication has no effect on the reference DNA standards.
	Instrument suitability	Good consistency of the assay performance on different qPCR instruments (e.g. HZSKBio® SHENTEK-96S, ThermoFisher ABI 7500, Bio-Rad CFX-96, Bioer FQD-96A, etc.)

Case Study—Residual Host Cell DNA Quantitation

CHO HCD Validation performance—

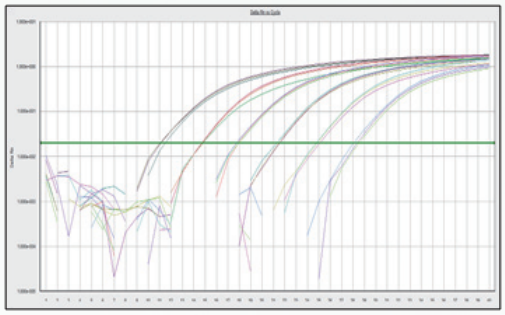
Standard curve: Amplification efficiency 96.6%, Correlation coefficient 0.999

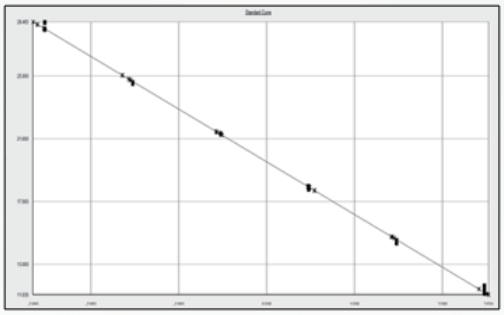




MDCK HCD Validation performance—

Standard curve: Amplification efficiency 98.2%, Correlation coefficient 0.999





Residual Host Cell Nucleic Acid Technical Service

1.Lab Service using standardized platforms

1)Residual host cell DNA size analysis and method validation:

• Integrated solution of SHENTEK® residual host cell DNA & RNA assay validation and sample testing services for various biological samples, for example, in-process samples, bulk and final products

2) Sample suitability test:

• Specific sample suitability test services using SHENTEK® sample preparation kits and host cell DNA & RNA quantitation kits

2.Customized Technical Service

1)Customized kit development for specific-species/sequence residual DNA or RNA assay:

• qPCR method establishment: Development of qPCR quantitation system, including DNA reference material, DNA extraction methods and specific PCR probe, primer and reagents for amplification and detection;

• Assay validation: Validation of linear range, accuracy, precision, quantitation limit, specificity and robustness in compliance with pharmacopeial requirements;

• Automated sample preparation: Development of automated protocols for DNA/RNA extraction and recovery validation using rHCDpurify® Sample Purification System.

2)Development of residual host cell DNA/RNA detection assay for microorganisms that is used for antibiotic fermentation:

• qPCR method development: Species/sequence-specific quantitative PCR assay development for different microorganisms used for antibiotic production, to ensure highly sensitive quantitation with LOQ up to fg/μL DNA level;

• Automated sample preparation: optimized for quantitative recovery on rHCDpurify® sample preparation system, and integrated workflow solution of sample-to-results system;

• Regulatory filing: Experience and knowledge of policies, procedures, and filing documents for customized business needs, with successful service cases for the EMA drug registration.

3.Consultation & Training Programs

1) Laboratory capability improvement: Integrated QC solutions from laboratory design to test proficiency, to ensure qualified lab capability for the QC needs at different project stages (e.g. program establishment, system certification or product registration);

2) Customized training program: Theoretical and experimental training programs that are designed to improve general qPCR assay capabilities, as well as specific test skills.