SHENTEK

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SHENTEK® Residual Host Cell DNA Size Analysis

Product introduction

Pharmaceutical regulatory organizations around the world recommended acceptable limits of residual DNA fragments to both the amount and size distribution, mostly not exceeding 10 ng/dose, with a size limit of 200 bp in length.

SHENTEK® Host Cell Residual DNA Size Analysis Kits are designed for quantitating different sizes of residual host cell DNA fragments, which usually target four fragment size ranges (< 100 bp, 100-200 bp, 200-500 bp, to > 500 bp) via DNA-specific qPCR assays. The kits are used in parallel with Residual Host Cell DNA Quantitation Kits to analyze extracted DNA from test samples using SHENTEK® Residual DNA Sample Preparation Kits.

This series of products cover a wide range of host cell lines, for example, E.coli, Pichia pastoris, CHO, Vero, MDCK, Sf9, Human, etc. The kits measure the quantity of residual host cell DNA fragments of different lengths at various stages throughout the manufacturing processes. The assay performance has been validated by multiple third-party laboratories, and complies with pharmacopeial requirements.

SHENTEK® Residual DNA Sample Preparation Kits

Residual Host Cell 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 (the magnetic particle loading capacity of the automated extraction is 50% higher than the manual extraction). Efficient and reproducible recovery have been validated in multiple complex sample mixture (high protein, high salt, llow pH, etc.).

Product Number	Product Name	Quantity
1104191	SHENTEK® Residual Host Cell DNA Sample Preparation Kit	100 Extractions

SHENTEK® Residual Host Cell DNA Size Analysis Kits

Species sequence-specific host cell residual DNA quantitation and fragment size assessment can be evaluated simultaneously on real-time PCR (qPCR) system. The pre-designed primers and probes target gene sequence of sufficient length, allowing for a set of amplicons that usually span four ranges: ‹100 bp. 100-200 bp, 200-500 bp, and ›500 bp.

Quantitation of cDNA fragment of different lengths in the samples were determined by a standard curve of DNA standard, and the sensitivity can reach fg/L level. The assay performance has been fully validated, including linear range, accuracy, precision, limit of quantitation and specificity, in compliance with the pharmacopoeia requirements. The kits contain quantitation standards for different sizes of DNA fragments, and are available with sample preparation reagents in the form of a SHENTEK® Residual DNA Sample Purification Kit, which is optimized for highly efficient residual DNA recovery from complex mixtures of proteins, buffers, and salts.

Product Number	Product Name	Size	rcDNA Fragment Size (bp)
1103170-2	SHENTEK® Residual CHO DNA Size Analysis Kit (2G)	4 × 100 Reactions	‹100/100+/200+/500+
1103171-2	SHENTEK® Residual E. coli DNA Size Analysis Kit (2G)	4 × 100 Reactions	‹100/100+/200+/500+
1103173	SHENTEK® Residual Human DNA Size Analysis Kit (2G)	4 × 100 Reactions	‹100/100+/200+/500+
1103174	SHENTEK® Residual Vero DNA Size Analysis Kit (2G)	4 × 100 Reactions	‹100/100+/200+/500+
1103175	SHENTEK® Residual MDCK DNA Size Analysis Kit	4 × 100 Reactions	‹100/100+/200+/500+
1103176	SHENTEK® Residual HEK293 DNA Size Analysis Kit	4 × 100 Reactions	‹100/100+/200+/500+
1103177	SHENTEK® Residual Sf9 Residual DNA Size Analysis Kit	4 × 100 Reactions	‹100/100+/200+/500+
1103178	SHENTEK® Residual PG13 DNA Size Analysis Kit	3 × 100 Reactions	100+/200+/500+
1103179	SHENTEK® Residual BHK DNA Size Analysis Kit	4 × 100 Reactions	‹100/100+/200+/500+
SK030307S-P	SHENTEK® Residual HPV18 E6/E7 DNA Size Analysis Kit	4 × 100 Reactions	E6:100+/200+ E7:100+/200+

Lab Service using standardized platform

1. **Residual host cell DNA size analysis and method validation**
   Integrated solution of SHENTEK® residual host cell DNA size analysis method development, 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 testing services with SHENTEK® sample preparation kits and host cell DNA size analysis kits.

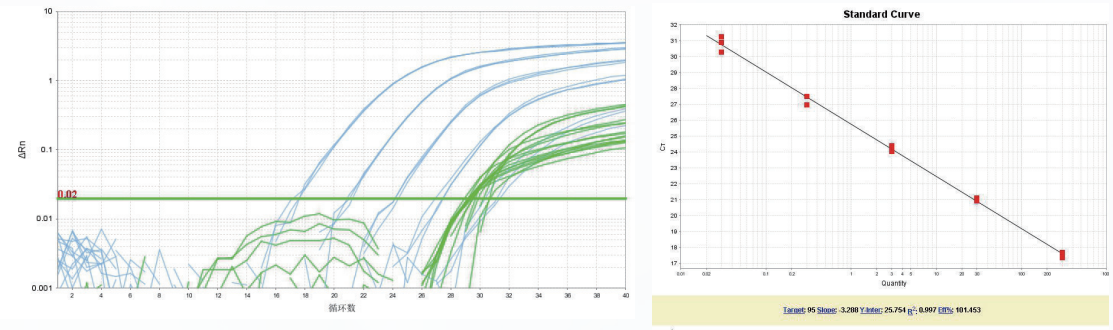
Validation of rcDNA Size Analysis

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

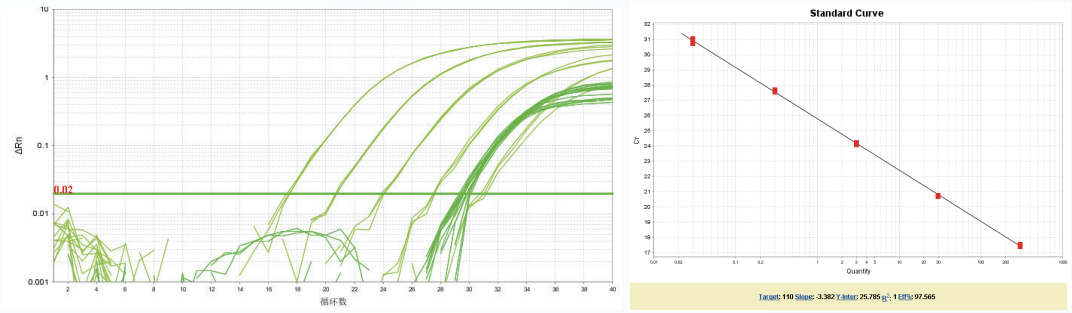
Parameters		Acceptance Criteria
Linear range		3 or 30 fg/μL to 300 pg/μL (Please refer to the specific User Guide); Correlation coefficient: $R^2 \geq$ 0.990;%CV (coefficient of variation) and %RE (relative error): ＜30%;
Accuracy		The intra-assay values met the following acceptance criteria: %CV: ＜ 30%;Recovery: 50%–150%;
Precision	Repeatability	The %CV of 10 intra-assay values at both high and low concentration levels were less than 30.0%;
	Intermediate precision	The %CV of 9 intra-assay values (3 concentration levels for each assay and 3 recoveries at each concentration level) were less than 30%;
Sensitivity		The detection limit met the specific acceptance criteria in the User Guider;
Quantitation Limit		The %CV of all replicates at the LOQ level are less than 30.0%;
Specificity		No cross-reactivity with the commonly used production cells, engineered bacteria and fungi, plasmid DNA, etc.
Robustness	Freeze-thaw stability	Stable assay performance for at least 4 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, Bloer LineGene 9600, etc.)

Case Study—CHO Host Cell DNA Size Analysis

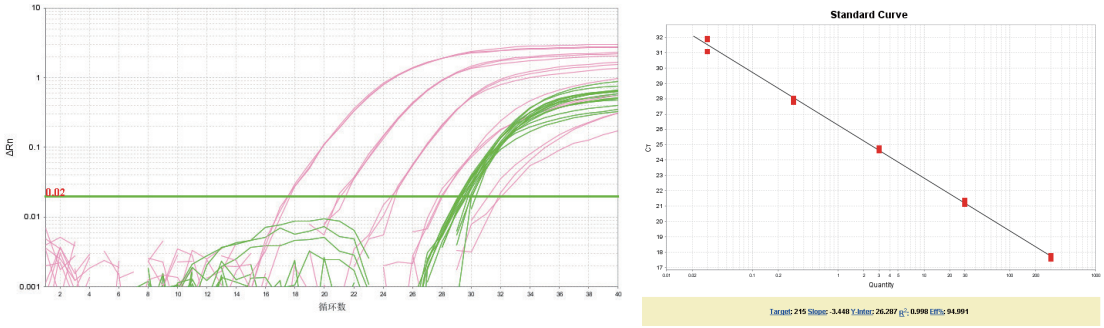
Validation performance - standard curve for 95 bp DNA size analysis:
Slope -3.288, amplification efficiency 101.5%, correlation coefficient 0.997



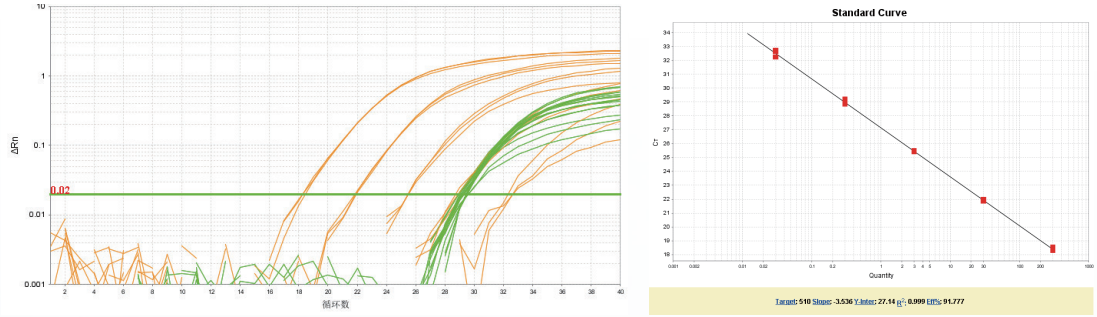
Validation performance - standard curve for 110 bp DNA size analysis:
Slope -3.382, amplification efficiency 97.6%, correlation coefficient 1.000



Validation performance - standard curve for 215 bp DNA size analysis:
Slope -3.448, amplification efficiency 95.0%, correlation coefficient 0.998



Validation performance - standard curve for 510 bp DNA size analysis:
Slope -3.536, amplification efficiency 91.8%, correlation coefficient 0.999



Customized Technical Service

Customized kit development for specific-species/sequence residual DNA size analysis and assay validation:

- 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 extraction and recovery validation for use with the rHCDpurify® Sample Purification System.

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