CHO residual DNA quantitation

- Highly sensitive quantitation of residual CHO DNA using real-time PCR
- Optimized sample preparation for quantitative DNA recovery from complex matrices
- Easy to use, with results in under 5 hours
- Specific to CHO DNA, no cross-reactivity with unrelated DNA
- Integrated system includes sample preparation kit, Applied Biosystems™ TaqMan® Assay and master mix, standard DNA, instrument, and software
- Protocol available for automated sample preparation

Introduction

The removal of host cell impurities is a critical step in the production of biopharmaceutical products. One impurity targeted for clearance during the purification process is residual DNA arising from host cells. In addition to potential safety issues associated with extraneous host cell DNA, the regulatory guidance for products produced in cell culture specifies that DNA content in the final product should be as low as possible, as determined by a highly sensitive method. Traditional methods of quantitating residual host cell DNA have been limited by laborious sample preparation protocols, lack of sensitivity and specificity, and slow time-to-results.

resDNASEQ Quantitative CHO DNA System

The Applied Biosystems™ resDNASEQ™ Quantitative CHO DNA System is a quantitative PCR (qPCR)-based system for the detection of residual DNA from the Chinese hamster ovary (CHO) cell line, a widely used cell line for production of biopharmaceutical products. The system overcomes the limitations of traditional methods by combining the proprietary high-recovery Applied Biosystems™ PrepSEQ™ Residual DNA Sample Preparation Kit and TaqMan Assay–based quantitation of host cell line DNA. The system enables rapid, specific quantitation of sub-picogram levels of CHO host cell DNA. Assay performance is very reliable, and quantitative results can be obtained in under 5 hours. The flexible sample throughput capacity of the assay allows for rigorous design and execution of DNA clearance studies. The accurate, reliable results allow for high-confidence tests across a broad range of sample types, from in-process samples to bulk drug substances.

Components of the PrepSEQ Residual DNA Sample Preparation Kit include:

- PrepSEQ Core Nucleic Acid Extraction Kit
- PrepSEQ Residual DNA Module

Components of the resDNASEQ Quantitative CHO DNA Kit include:

- TaqMan primer and probe mix
- Environmental master mix
- CHO genomic DNA standard
- Negative control

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Real-time PCR for highly sensitive quantitation

The resDNASEQ Quantitative CHO DNA System enables highly sensitive detection of CHO DNA, allowing the use of small sample volumes to generate accurate results. The broad linear range provided by TaqMan Assays allows testing of samples containing variable levels of CHO DNA, such as in-process samples that may contain higher amounts of DNA and bulk drug substances that would contain very low amounts of DNA, to be analyzed in the same assay. Figure 1 demonstrates the range and sensitivity of the assay. Linearity is demonstrated by analysis of CHO cell standard DNA ranging from 0.3 ng to 3 fg.

PrepSEQ sample preparation kit

The PrepSEQ Residual DNA Sample Preparation Kit is optimized for highly efficient DNA recovery from complex mixtures of proteins, buffers, and salts. Quantitative and consistent recovery of DNA can be obtained from challenging matrices, including samples that have high protein or salt concentration, or low pH.

Automation of the sample preparation procedure on the Thermo Scientific™ KingFisher™ Flex instrument provides an easy-to-use high-throughput workflow. Using the automated system, residual DNA can be isolated from up to 24 test samples (in triplicate) in 5 hours.

Table 1 demonstrates the 90% or greater recovery from a typical antibody drug substance test sample that had been spiked with 100, 10, 1, or 0.1 pg CHO standard DNA in triplicate. The coefficient of variation (CV) for each of the concentrations demonstrates the high reproducibility of the automated sample preparation protocol.

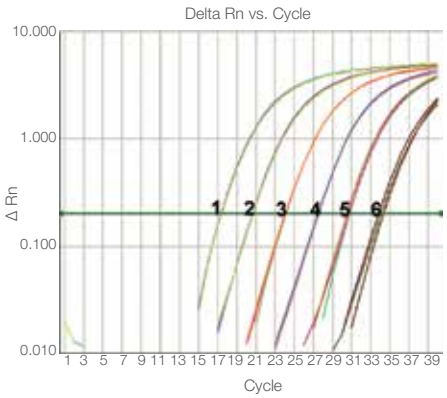
Easy to use, results in under 5 hours

Starting with DNA extracted from in-process purification and drug substance samples, real-time qPCR is utilized to compare DNA amounts in test samples to a standard curve generated with known amounts of purified CHO cell standard DNA. The easy workflow of the resDNASEQ Quantitative CHO DNA System consists of sample preparation, assay setup, and instrument run and data analysis—all of which can be performed in under 5 hours (Figure 2).

Specific to CHO DNA, no cross-reactivity with unrelated DNA

The target of the assay was designed to be highly specific so that it only detects a hamster-specific region of a multicopy genetic element. This region was selected using extensive bioinformatic analysis of multiple related and unrelated species. Testing of assay performance confirmed that the assay is specific to hamster DNA and is unaffected by the presence of as much as 100 ng of unrelated DNA in a test sample (Figure 3).

A



B

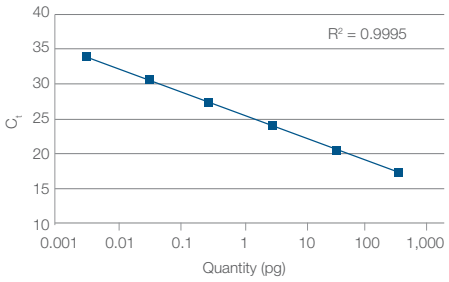


Figure 1. Dynamic range and sensitivity. (A) Amplification plots generated from running the assays with 10-fold serial dilutions from 0.3 ng to 3 fg of CHO genomic DNA that was purified from CHO cell line K1. **(B)** Standard curve and correlation coefficient.

Table 1. High DNA recovery rate from a typical antibody drug substance.

Spiked DNA quantity	Quantity recovered (pg)			Average recovery	CV
100 pg	105	133	123	120%	12%
10 pg	10.2	10.9	11.5	109%	6%
1 pg	1.1	1.1	1.2	110%	5%
0.1 pg	0.09	0.10	0.08	90%	7.9%

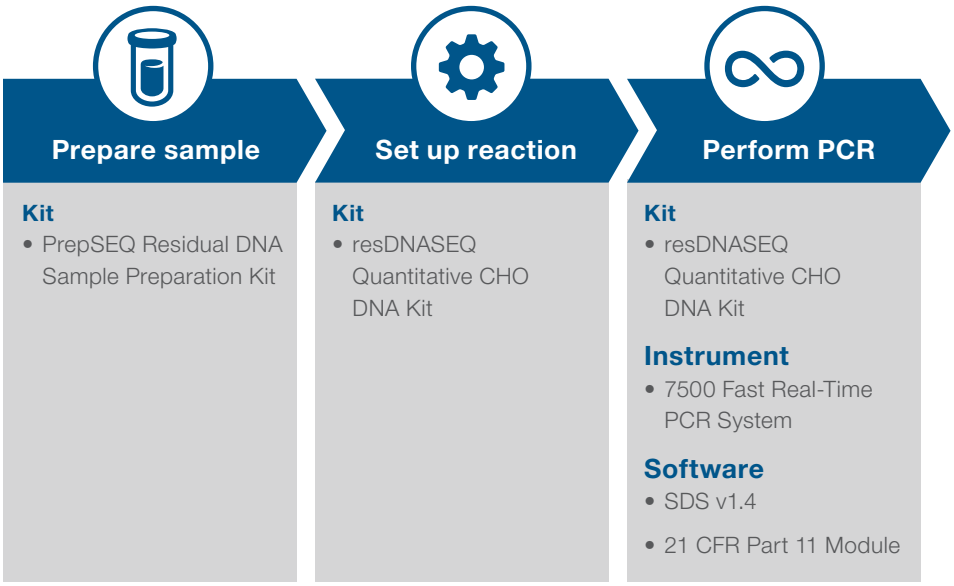


Figure 2. Workflow of the resDNASEQ Quantitative CHO DNA System.

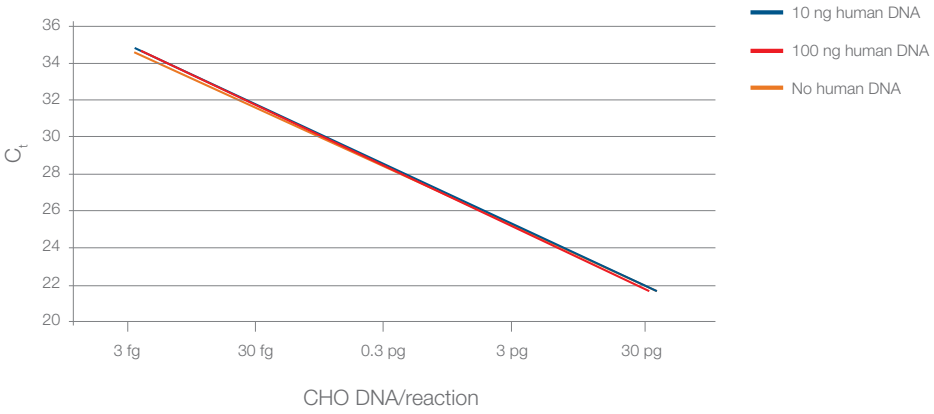


Figure 3. Specificity of the assay in the presence of human DNA. The standard curve generated with purified CHO genomic DNA in the absence of human DNA was overlaid with the curves generated in the presence of 10 ng human DNA and 100 ng human DNA.

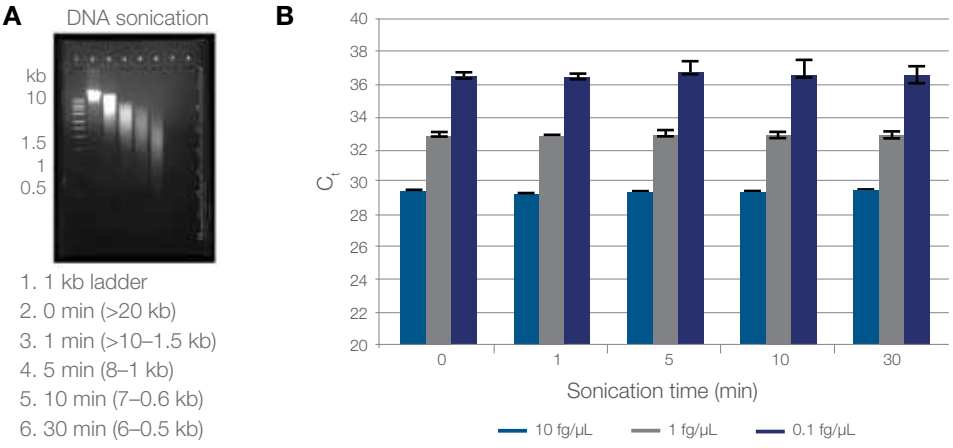


Figure 4. Consistent performance across fragmented and high molecular weight DNA. **(A)** Agarose gel showing the high molecular weight control DNA (0 min sonication) and CHO cell line DNA that was sonicated for various times. **(B)** The C_t values obtained with different concentrations of the fragmented DNAs (shown in **A**) are comparable to those of the high molecular weight control DNA.

Consistent performance even with fragmented DNA

For the most accurate quantitation of residual CHO DNA, the assay results must be unaffected by the size of the DNA molecules present in the test sample. For example, the assay must perform as consistently with DNA from in-process samples that might contain some amount of sheared or fragmented genomic DNA as it would with unfragmented, high molecular weight DNA. To test the effect of DNA fragment size on assay performance, a fragmentation model system was created where high molecular weight CHO genomic DNA was fragmented to lower molecular weight DNA by sonication for varying times. Figure 4 demonstrates that the C_t values for the reactions with the sonicated lower molecular weight DNA were comparable to those of the unsheared high molecular weight DNA. These results demonstrate that consistent performance was obtained with the kit regardless of DNA molecular weight.

Summary

The resDNASEQ Quantitative CHO DNA System is the first integrated real-time qPCR system for the quantitation of residual DNA, with optional automated sample preparation. The high recovery of the PrepSEQ sample preparation kit combined with the assay's high sensitivity and specificity should enable accurate and reproducible quantitation of CHO residual DNA from diverse sample types, such as in-process purification samples, bulk drug substances, or final products. Using the automated system, residual DNA can be isolated from up to 24 test samples (in triplicate) in 5 hours.

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Ordering information

Product	Quantity	Cat. No.
resDNASEQ Quantitative CHO DNA Kit	100 reactions	4402085
resDNASEQ Quantitative CHO DNA Kit with PrepSEQ Residual DNA Sample Preparation Kit	100 reactions	4413713
Sample preparation and automation		
PrepSEQ Residual DNA Sample Preparation Kit	100 reactions	4413686
Pharma KingFisher Flex 96 Deep-Well Magnetic Particle Processor	1 instrument	A31508
Real-time PCR system		
7500 Fast Real-Time PCR System	1 instrument	4365464
Software		
AccuSEQ Real-Time PCR Software	1 license	4443420
Service		
7500 Fast IQ/OQ Service		4365572

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