CHO Residual DNA Quantitation

- Real-Time quantitative PCR system for highly sensitive residual CHO DNA quantitation
- Optimized sample preparation for quantitative DNA recovery from complex matrices
- Easy to use; results in under five hours
- Specific to CHO DNA, no cross-reactivity with unrelated DNA
- Integrated system includes sample preparation, 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 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 PrepSEQ™ sample preparation kit, and TaqMan® Residual DNA Detection Kit-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 five hours. The flexible sample throughput capacity of the assay allows for rigorous DNA clearance study design

and execution. The accurate, reliable results allow for high-confidence tests across a broad range of sample types, from in-process samples to bulk drug substance.

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 System include:

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

Real-Time PCR for Highly Sensitive Quantitation

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

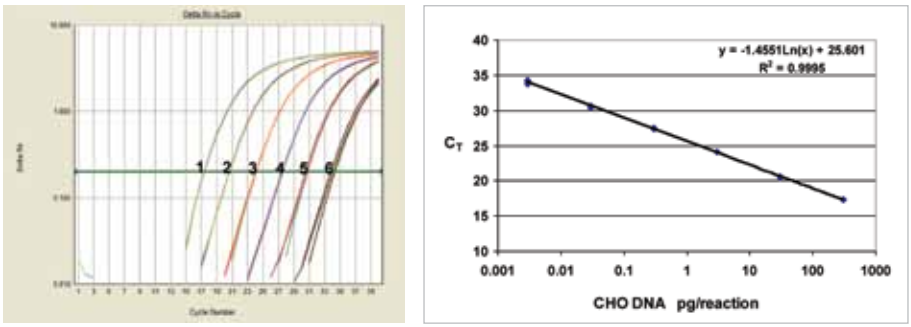

Figure 1. Dynamic Range and Sensitivity. Left panel shows the amplification plots generated from running the assays with a 10-fold serial dilution from 0.3 ng to 3 fg of CHO genomic DNA that was purified from CHO cell line K-1. On the right is the standard curve along with the slope and correlation coefficient.

TABLE 1. EFFICIENCY GAINS THROUGH AUTOMATED SAMPLE PREPARATION

Spiked DNA Quantity	Picograms of DNA Recovered			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%

High DNA recovery rate from a typical antibody drug substance.

PrepSEQ™ Sample Preparation Kits

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 concentration, low pH, or high salt. Automation of the sample preparation procedure on the MagMax™ Express 96 instrument provides an easy-to-use workflow for high-throughput sample preparation. 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, or 1 pg CHO standard DNA in triplicate. The %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 set-up, and instrument run and data analysis—all of which can be performed in under five 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).

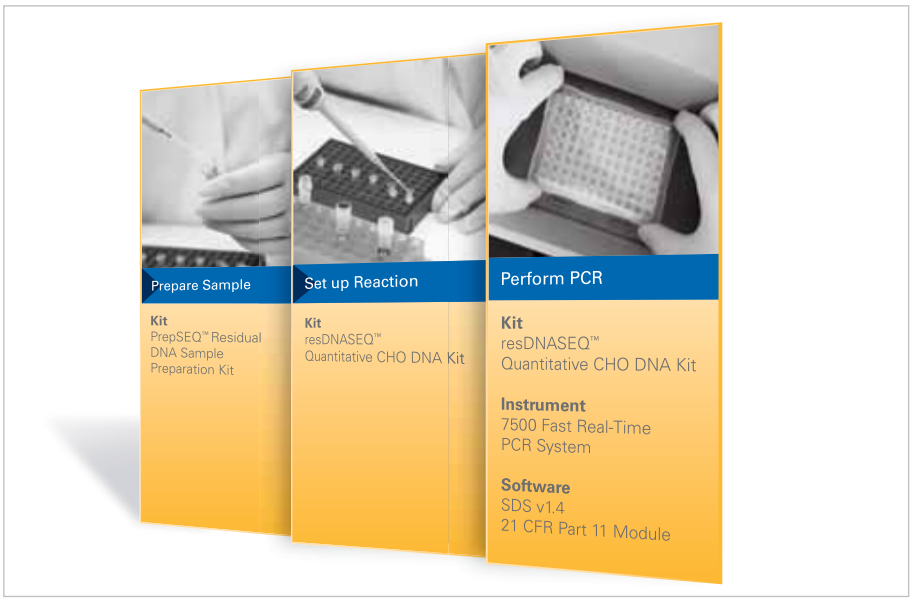

Figure 2. Workflow of the resDNASEQ™ Quantitative CHO DNA System.

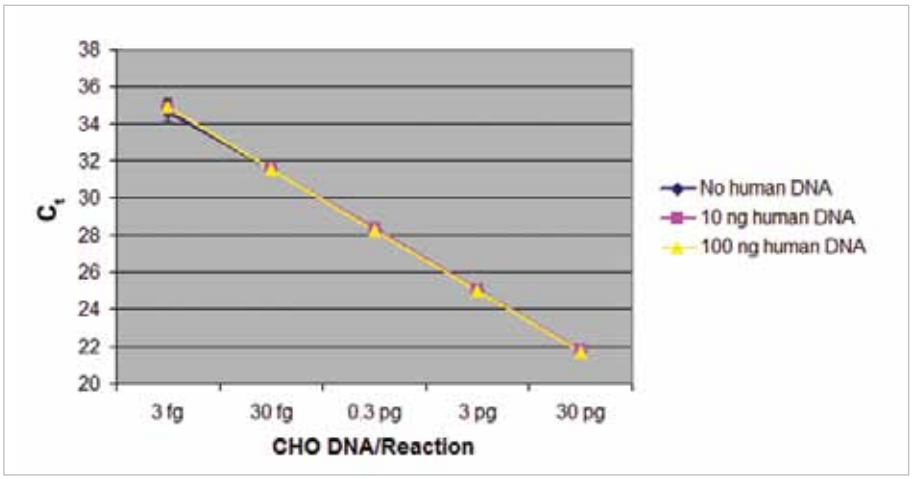

Figure 3. Specificity of the Assay in the Presence of Human DNA. The standard curve generated with purified CHO genomic DNA in the absence of human DNA (blue diamonds) was overlaid with the curves generated in the presence of 10 ng human DNA (pink squares) and 100 ng human DNA (yellow triangles).

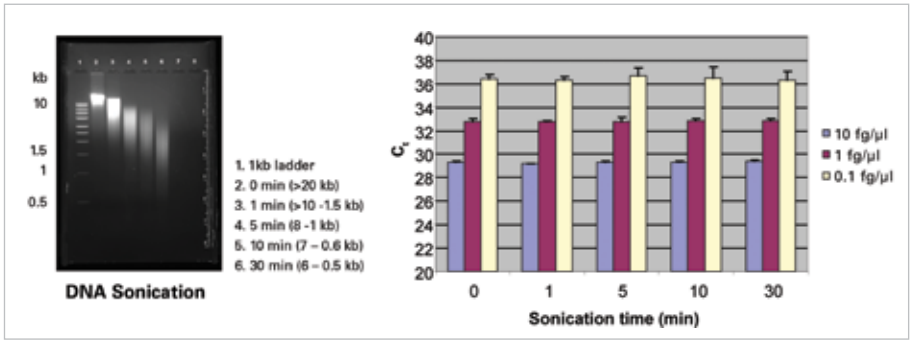

Figure 4. Consistent Performance Across Fragmented and High Molecular Weight DNA. On the left is an agarose gel showing the high molecular weight control DNA (0 min sonication) and CHO cell line DNA that was sonicated for various times. The right panel demonstrates the C_t values obtained with different concentrations of the fragmented DNAs (shown in the left panel) being comparable to those of the high molecular weight unfragmented 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 low molecular weight DNA by sonication for varying times. Figure 4 demonstrates that the threshold cycle (C_t) values for the reactions with the sonicated low molecular weight DNA were comparable to those of the undigested high molecular weight DNA. Note that the C_t is the value that is used to quantify the unknown samples. These results demonstrate that consistent performance was obtained with the kit irrespective 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 procedure combined with the assay's high sensitivity and specificity should enable accurate and reproducible CHO residual DNA quantitation from diverse sample types, such as in-process purification samples, bulk drug substance, or final product.

ORDERING INFORMATION

Description	P/N
resDNASEQ™ Quantitative CHO DNA Kit + PrepSEQ™ Residual DNA Sample Preparation Kit 100 rxn, without Protocol and Quick Reference Card	4413713
resDNASEQ™ Quantitative CHO DNA kit 100 rxn, without Protocol and Quick Reference Card	4402085
PrepSEQ™ Residual DNA Sample Preparation Kit 100 rxn, without Protocol and Quick Reference Card	4413686
resDNASEQ™ CHO genomic DNA standard 100 rxn	4403965
MagMAX™ Express 96 DW, Applied Markets	4400079

For sample preparation automation protocol, contact your AB Field Application Specialist.

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