

Host Cell DNA

Residual DNA Quantitation

ResidualQSearch™ **CHO DNA Quantitation Kit**

Used for the quantitative analysis of residual DNA derived from cell-culture-based pharmaceuticals.

A quantitative CHO DNA standard material is included in the product.

Real-time quantitative PCR(qPCR) using TaqMan probe

Detection Limit : 0.3 fg/reaction

Rapid: The results are within 1~2 hours

Compliance with Korea MFDS, WHO, EP and JP

UDG system: prevention of carry-over contamination



Introduction

CellSafe *ResidualQSearch™* CHO DNA Quantitation Kit detects Chinese Hamster Ovary (CHO) cell DNA using real-time PCR. The primer and probe mix is included in *ResidualQSearch™* CHO DNA Quantitation Kit. The FAM-labeled probe and primer which are specific to CHO genomic DNA are used to detect target-specific regions only among multicopy genetic elements. The presence of CHO cell gDNA can be easily confirmed as DNA from other species does not amplify. In addition, CHO cell DNA can be quantified using the standard curve generated with the CHO DNA Standard included in this product.

Sample Preparation

The preparation of sample to be tested with this Kit is crucial for measuring the amount of residual DNA from CHO host cells. Appropriate treatment is required depending on the type of samples (e.g., high concentration proteins, antibodies and vaccines, etc.). For instance, in the presence of high-concentration proteins, proteinase treatment may be necessary and in cases where inhibitory substances reduce DNA recovery, commercially available extraction kits such as magnetic bead or column-based kits can be used to prepare for the samples.*It is recommended to prepare 3 types of samples as below.

- 1) The Sample to be tested
- 2) The Sample spiked with CHO gDNA
 - : to confirm CHO DNA extraction Recovery
- 3) Sample-free buffer solution
 - (e.g. PBS or buffer solution used in sample preparation)
 - : To check for CHO DNA contamination during the extraction process

Storage

Store at -20°C. (Frequent freezing and thawing might lead to deterioration in product quality. Aliquoting the CHO DNA Standard is recommended for accurate quantification.)
Store separately packed DNA Dilution Buffer at 2-8°C.

The Highest Sensitivity in Residual DNA Testing

ResidualQSearch™ CHO DNA Quantitation Kit has the high sensitivity of 0.3fg/reaction to detect target genes. In addition, the CHO DNA standard included in the Kit was validated for linearity from 0.3ng/μl to 0.3fg/μl. CHO genomic DNA was serially diluted 10-fold and tested, and as shown in the figure1, *ResidualQSearch™* CHO DNA Quantitation Kit was verified to have high sensitivity, quantifying up to 3fg/reaction.

* LOD : 0.3fg/reaction, LOQ : 3fg/reaction

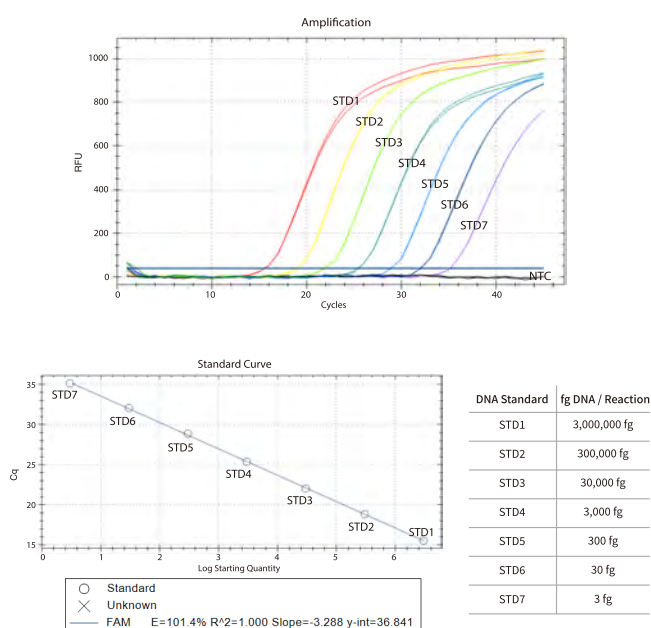


Figure 1 CHO standard DNA serial dilution & Standard Curve

Specific to CHO DNA

ResidualQSearch™ CHO DNA Quantitation Kit detects target genes that are common and specific to all CHO cells using real-time Polymerase Chain Reaction (qPCR). It specifically detects the hamster-specific region of the multicopy genetic elements and does not amplify the microbial and animal cell DNA of other species. The performance of the Kit is not affected even when 3000pg of unrelated DNA is present in the sample.

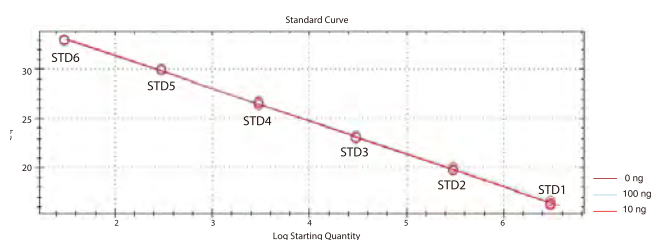
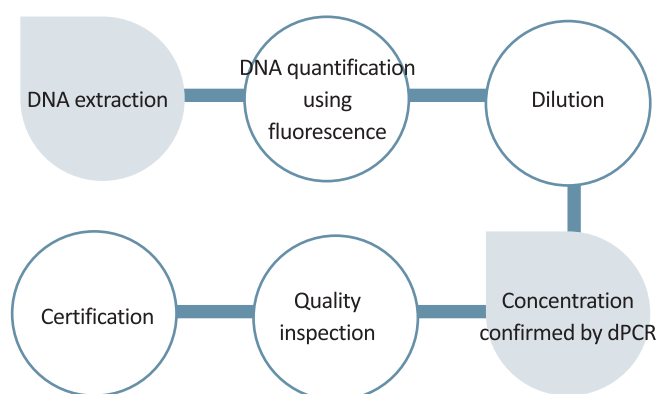


Figure 2 Human gDNA Spiked specificity test

The production process of CHO Standard DNA



Stable assay result even with fragmented DNA

For the accurate quantitation of CHO residual DNA, the assay results should not be affected by the size of the DNA in the sample. Fragmentation of DNA by shear forces is common in the production process of biopharmaceuticals. Therefore, it is necessary to verify that sufficient recovery is achieved even when the DNA fragments in the sample are too small. To confirm that the size of DNA fragments does not affect the test results, large molecular weight DNA was sonicated for various strengths of energy to break it into low molecular weight fragments, and the results were compared to those obtained from intact high molecular weight DNA.

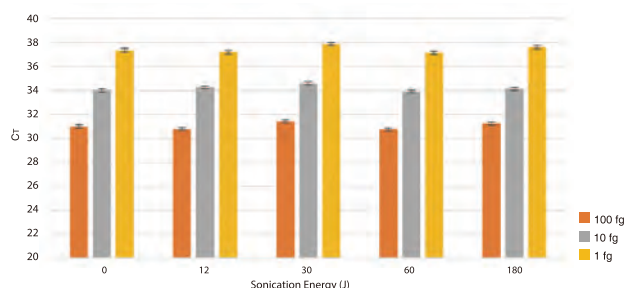
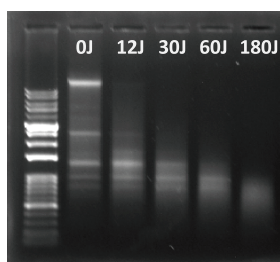


Figure 3 Consistent performance across fragmented DNA

The *ResidualQSearch™* CHO DNA Quantitation Kit includes quantitative DNA for measuring residual DNA in samples, as well as rehydration buffer. Several quality inspections are conducted to ensure accurate quantification of the standard substance.

The CHO-K1 cell line is purchased from ATCC, and is continuously maintained and periodically tested for mycoplasma. The DNA is extracted, quantified using fluorescence, and diluted to the desired concentration. The accurate DNA concentration is confirmed using digital PCR, and the product undergoes quality control before being released for shipment. dPCR-verified CHO standard DNA ensures the reliability of the test.

No false positive result

The probe of *ResidualQSearch™* CHO DNA Quantitation Kit is specifically designed for CHO genomic DNA so it does not cause false positives.

If amplification occurs in NTC (No Template Control), it can affect the standard curve and make it difficult to accurately quantify CHO DNA, resulting in low reliability of the results. The *ResidualQSearch™* CHO DNA Quantitation Kit can quantify trace amounts of CHO DNA because there is no false positive in NTC (No Template Control).

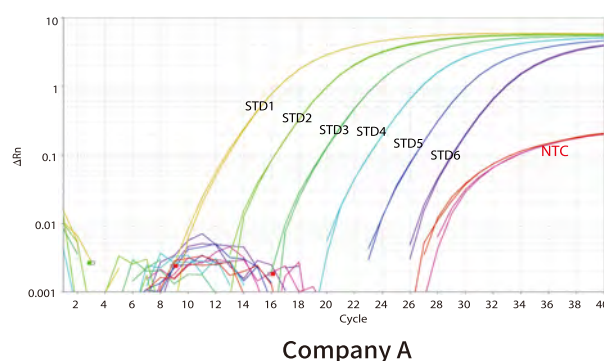
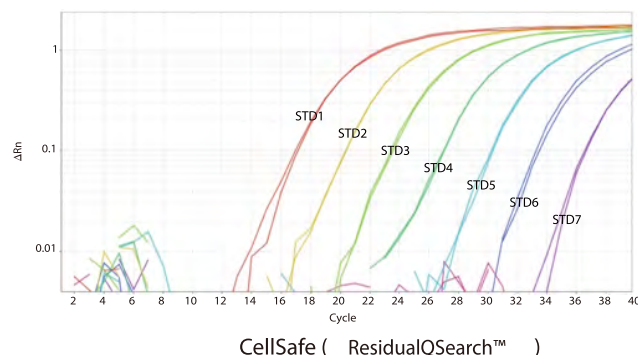


Figure 4 Comparison of residual DNA quantitation kit

