ResidualQSearch™ Plus

CHO DNA Quantitation Kit

Version:2.0

Latest update: 2024.02.01

Instruction Manual

Cat. No. CHOP-100

Research Use Only. Not for Use in Diagnostic Procedures.

Contents

1. Product Information

Introduction

The ResidualQSearch™ Plus CHO DNA Quantitation Kit is designed for the quantification of Chinese Hamster Ovary (CHO) cell DNA remaining in the pharmaceutical production process using real-time quantitative polymerase chain reaction (qPCR) with probes. The primer and probe mix in the Kit includes two types of probes. One probe labeled with FAM is specifically designed for CHO cell genomic DNA and the other probe labeled with HEX is designed for internal control DNA to confirm success of PCR reaction. The primers are designed to be specific to Hamster genomic DNA, allowing the detection of the hamster-specific region of the multicopy genetic element using these primers. DNA from other microbial species and animal cells is not amplified by the ResidualQSearch™ Plus CHO DNA Quantitation Kit.

This kit facilitates the rapid quantification of residual DNA from CHO host cells in pharmaceuticals within 3 hours.

Kit Specificity

The ResidualQSearch™ Plus CHO DNA Quantitation Kit detects CHO cell genomic DNA simply, reliably, and rapidly. To quantify the amount of CHO cell genomic DNA, the assay utilizes the polymerase chain reaction (PCR) to amplify a target unique to a wide variety of CHO cells. The kit can amplify almost all types of CHO cell lines. Importantly, the kit does not detect DNA from other genera or cell lines.

Kit Sensitivity

The sensitivity of the PCR using the ResidualQSearch™ Plus CHO DNA Quantitation kit is 0.3fg of CHO genomic DNA per reaction. The sensitivity of the assay in real culture samples depends on the quality of the sample preparation.

Prevention of carryover contamination

Take precautions to minimize the potential for carryover of nucleic acids from one experiment to the next. Use separate work areas and pipettes for template preparation and PCR setup steps. Use the PCR grade water delivered with the kit, aerosol-preventive filter tips and gloves.

The 2X qPCR master mix contains dUTP instead of dTTP. When dUTP replaces dTTP in PCR amplification, UNG treatment (Uracil-N-glycosylase, HiSense™ UDG heat labile, Cat.No.SCU-100, not provided in this kit) can prevent the subsequent reamplification of dU-containing PCR products. UNG acts on single- and double-stranded dU-containing DNA by hydrolysis of uracil-glycosidic bonds at dU-containing DNA sites. With this strategy, carryover contamination is eliminated, while the template DNA (DNA containing T) remains intact.

2. Contents and Storage

Materials Provided

Label	Cap	Content
2X qPCR Master Mix	Blue	1.5 ml
Primer and Probe Mix	Amber	200 µl
CHO DNA Standard, 30ng/µl	Green	40 µl
DNase Free Water	White	1.2 ml
DNA Dilution Buffer	-	20 ml

Storage

Store at -20°C.

Note:

1) Repeated thawing reduces the quality of product.

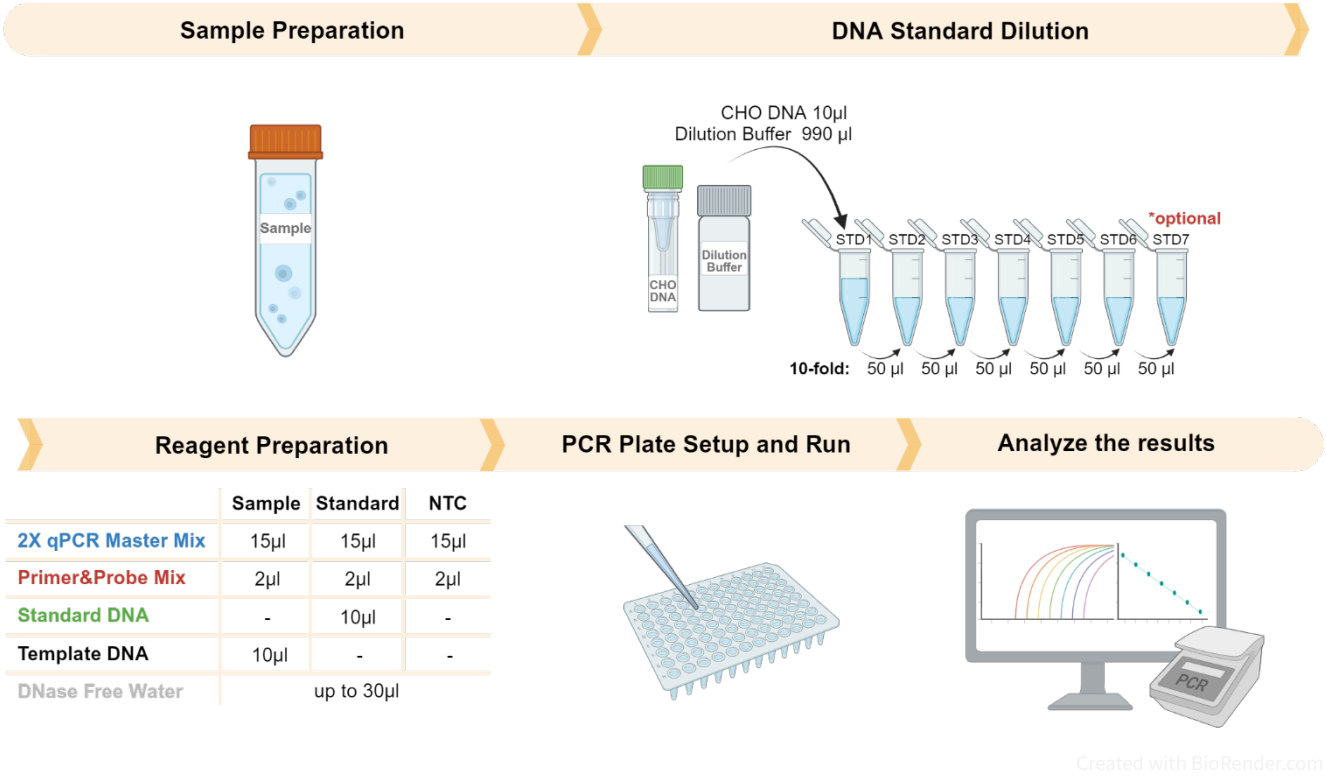
2) If frequent freezing and thawing are necessary, aliquot the products and use them in order.

3) Check the label on the product for details.

4) DNA Dilution Buffer can be stored in the refrigerator for convenience.

3. Test protocol

Workflow



	Sample	Standard	NTC
2X qPCR Master Mix	15µl	15µl	15µl
Primer&Probe Mix	2µl	2µl	2µl
Standard DNA	-	10µl	-
Template DNA	10µl	-	-
DNase Free Water	up to 30µl		

Prepare the Template (Sample)

The preparation of the samples for testing with this kit is crucial to measure the residual DNA quantity from CHO host cells. Given the variability in sample types (e.g., high concentrations of proteins or antibodies, vaccines, etc.), appropriate processing steps are necessary based on the specific characteristics of each sample. In the presence of high protein concentrations, procedures such as proteinase treatment are required. For other inhibitory substances that may reduce DNA recovery, commonly used DNA extraction kits employing magnetic bead can be utilized for sample preparation.

It is recommended to prepare samples in the following three categories and perform the DNA extraction:

1.Test sample

2.Test Sample spiked with CHO gDNA : For checking DNA extraction recovery

3.Buffer solution without the test sample (e.g., PBS or buffer solution used in sample preparation)

To verify CHO DNA contamination during the DNA extraction process

Prepare for qPCR

The standard curve and test samples are processed on separate workstations, involving the use of distinct pipettes. To prevent false positive results, PCR-grade water and aerosol-preventive filter tips provided in the kit are used, and gloves are worn during the experiment.

1. Prepare the CHO DNA Standard by diluting it as indicated in the table below.

It is crucial to thoroughly mix the mixture before each dilution step. After spinning down the tube before each dilution step, proceed to the next dilution step.

DNA Standard	Dilution Method	Concentration (fg/µl)
STD1	10 µl DNA Standard + 990 µl DNA Dilution Buffer	300,000
STD2	50 µl STD1 + 450 µl DNA Dilution Buffer	30,000
STD3	50 µl STD2 + 450 µl DNA Dilution Buffer	3,000
STD4	50 µl STD3 + 450 µl DNA Dilution Buffer	300
STD4	50 µl STD4 + 450 µl DNA Dilution Buffer	30
STD6	50 µl STD5 + 450 µl DNA Dilution Buffer	3
STD7*	50 µl STD6 + 450 µl DNA Dilution Buffer	0.3

* Typically, STD1 to STD6 are used, and for higher sensitivity, it is recommended to include STD7 (0.3fg/ µl)

2. Prepare the reaction mixture as indicated in the table below

Reaction Components		Sample Reation	CHO DNA Standard Reaction	No Template Control (NTC) Reaction
Label	Cap			
2X qPCR Master Mix	Blue	15 µl	15 µl	15 µl
Primer and Probe Mix	Amber	2 µl	2 µl	2 µl
Template DNA	-	10 µl	-	-
Standard DNA	-	-	10 µl	-
DNase Free Water	White	Up to 30 µl		
Final volume		30 µl	30 µl	30 µl

3. Set up the qPCR machine program as follows.

Steps & Cycle			Temp(℃)	Time
Pre Heat			95	5 min
PCR	40Cycles (STD 1-6) Or 45 Cycles (STD 1-7)	Denature	95	20 sec
		Anneal	60	30 sec
		Extend*	72	30 sec
		*Acquisition CHO DNA – FAM (510 nm), Green channel Internal Control DNA – HEX or VIC (595~610nm), Yellow channel		

4. Results

The presence of CHO cell gDNA in the sample is detected through an increasing fluorescence signal in the FAM channel. False-negative results (PCR reaction inhibition caused by sample composition) may occur depending on the sample type, in which case no fluorescent signal will be observed.

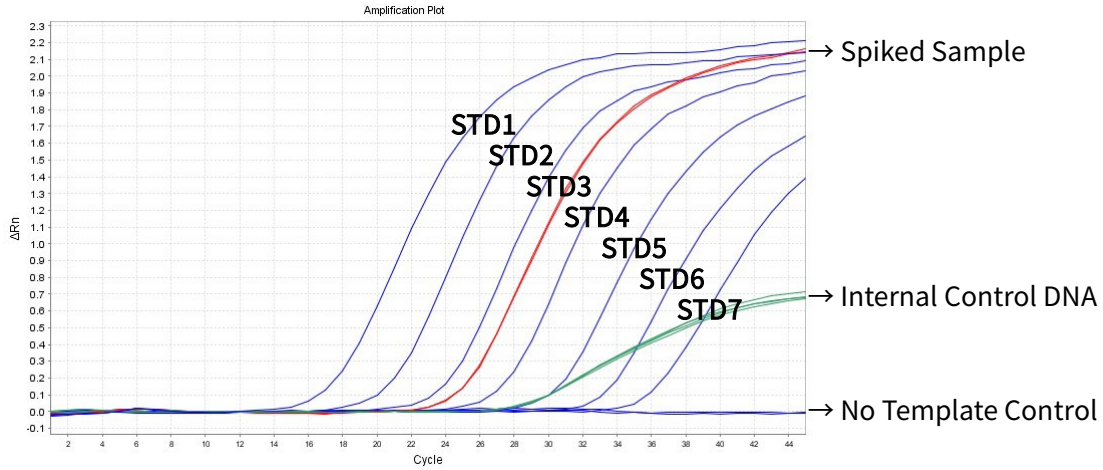
To quantify the amount of CHO cell gDNA in the sample, a standard curve for FAM can be generated using the CHO DNA Standard (30 ng/µl) provided in this kit and DNA Dilution Buffer. Refer to the table below, inputting values for STD1 to STD6 or STD1 to STD7 and use the STD Curve to determine the amount of CHO cell DNA in the sample.

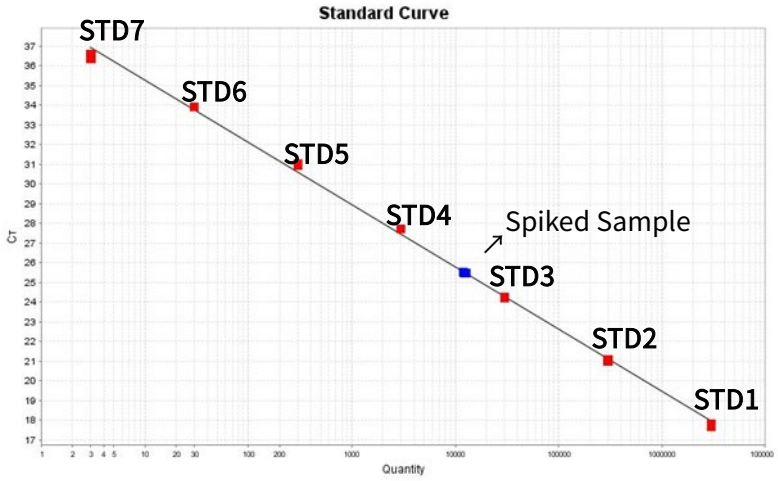
Quantify the CHO gDNA value by substituting the experimental results (Ct values) of the sample into the STD curve. When determining the final CHO gDNA value, reference can be made to the quantification value of the sample spiked with CHO gDNA and the quantification value of the buffer solution without the sample.

DNA Standard	DNA Concentration (fg/reaction)
STD1	3,000,000
STD2	300,000
STD3	30,000
STD4	3,000
STD4	300
STD6	30
STD7*	3

* Typically, STD1 to STD6 are used, and for higher sensitivity, it is recommended to include STD7 (0.3fg/µl)

Amplification Result and Standard Curve





Calculating the amount of residual DNA

1. Plot the log quantity of DNA of the Standard reactions versus the Ct.

2. Calculate the slope and the intercept.

Using these values and the following equation, calculate the quantity of Residual CHO DNA in sample reaction.

$$\text{DNA Quantity} = 10^{\frac{Ct-b}{m}}$$

Ct = cycle threshold of the Sample

b = y-intercept of the line for the Standard reactions

m = slope of the line for the Standard reactions