Instructions for Use of CHO Residual DNA Detection Kit (qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- CH001

Introduction

The CHO Residual DNA Detection Kit (qPCR) is designed for the quantitative detection of residual CHO DNA content in intermediates, semi-finished products and finished products of various biological products. This kit adopts the principle of the Taqman probe to quantitatively detect CHO residual DNA in samples. The kit is a rapid, specific and reliable device, with the minimum detection limit reaching fg level.

The CHO DNA quantitative standard provided in the kit can be traced to the national standard.

The assay range of this kit is: 3 fg/μL ~ 3×10^5 pg/μL.

Specification

100 Reactions

Kit components

Table 1: Kit components and storage conditions

Components	Specification	Storage conditions
2×qPCR Reaction MIX	1.6 mL × 1 vial	-20℃ or below, protected from light
CHO Primer&Probe MIX	550 μL × 1 vial	
Quantitative standard 1 (300 pg/μL)	300 μL × 1 vial	
Quantitative standard 2 (30 pg/μL)	300 μL × 1 vial	
Quantitative standard 3 (3 pg/μL)	300 μL × 1 vial	
Quantitative standard 4 (0.3 pg/μL)	300 μL × 1 vial	
Quantitative standard 5 (0.03 pg/μL)	300 μL × 1 vial	
Quantitative standard 6 (0.003 pg/μL)	300 μL × 1 vial	
DNA diluent	1 mL × 3 vials	

Shelf life

The shelf life is 12 months when stored at specified storage conditions.

Apparatus to be prepared by the user

Fluorescent quantitative PCR system

1.5 mL sterile centrifuge tube

Pipettes of 1000 μL, 100 μL, and 10 μL specifications

Sterile low-attachment pipette tips with cartridge, 1000 μL, 100 μL, and 10 μL specifications

Sterile, enzyme-free 8-strip PCR tubes or 96-well qPCR plate

Applicable models (including but not limited to)

- ABI QuantStudio 3 qPCR System
- Roche LC96 Real-Time PCR System
- ABI 7500 Real-Time PCR System
- Bio-Rad CFX Opus96 Real-Time PCR System
- RocGene ArchimedTM X Real-Time PCR System

Test procedures

Preparation and addition of qPCR reaction solution

1. Calculate the required number of reaction wells based on the numbers of standards and samples to be tested (generally, 3 replicate wells will be required for each sample):

Number of reaction wells = (6 sets of standards + 1 no template control (NTC) + test samples) × 3.

2. Calculate the total volume of CHO qPCR MIX required for this analysis based on the number of reaction wells.

Volume of CHO qPCR MIX = (number of reaction wells + 2 or 3) × 20 μL (including volume loss of 2 or 3 wells during operation).

3. Place each reagent on ice to thaw, gently shake to mix well, and prepare CHO qPCR MIX according to Table 2.

Table 2. Preparation of CHO qPCR MIX

Components	Volume required for single reaction
2×qPCR Reaction MIX	15 μL
CHO Primer&Probe MIX	5 μL

4. Place each reagent on ice to thaw, gently shake to mix well, and load to wells according to Table 3 (total volume: 30 μL).

Table 3. Examples of loading to each reaction well

Template	Volume for the template	Volume of CHO qPCR MIX required
Standards	10 μL each for quantitative standards 1 ~ 6	20 μL

No template control (NTC)	10 μL for each DNA diluent buffer	20 μL
Test sample	10 μL for each test sample	20 μL

5. The analysis can be carried out using sterile and nuclease-free 8-strip PCR tubes or 96-well plates. Bubbles must be removed from the reaction system. Centrifuge to allow the liquid to gather at tube bottom for reaction.

qPCR reaction program and parameter setting

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Set up the reaction program:

2. Create an experimental reaction plate, and click on "Select Fluorophores" and select fluorescent FAM. Select sample wells in the reaction plate chart, select "Unknown" in the "Sample Type" dropdown menu, check fluorescent FAM, and name the Target Name as "CHO", then input the number of replicate wells and Sample Name for each sample.

Select standard curve wells in the reaction plate chart, select "Standard" in the "Sample Type" dropdown menu, check the fluorescence FAM, and name the Target Name as "CHO", then input the number of replicate wells and Sample Name for each dilution gradient. Assign values of 300000, 30000, 3000, 300, 30, and 3 (in pg/μL), respectively, to the "Concentration" column of STD1, STD2, STD3, STD4, STD5, and STD6.

3. Click "Start Run" on the "Run" interface to perform PCR analysis.

Stage1	Contamination digestion	Reps: 1	50℃	2min
Stage2	Pre-denaturation	Reps: 1	95℃	20s
Stage3	Cyclic reaction	Reps: 40	95℃	3s
			60℃	30s

Set the collection of fluorescence in Stage 3 of the program (at 60℃ for 30 seconds);

qPCR result analysis

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Click "Quantitation" on the data analysis window to read the slope, intercept, amplification effect, and R^2 of the standard curve.

2. In the "Quantitation Data" window, the RCL detection values (in copies/μL) of NTC and test samples can be read in the "SQ Mean" column.

3. For NTC, the result should be N/A, or the Ct value should be greater than the mean Ct value of the lowest concentration on standard curve.

Test procedures

1. This kit is for *in vitro* detection only, and may not be used for clinical diagnosis.
2. The kit must be used within the shelf life.
3. All components in the kit should be used after thawing in a low-temperature environment.
4. The optimal assay results may only be achieved by strictly following the instructions and using only the reagents provided in the kit.
5. Please timely replace pipette tips when loading different samples and performing different steps, so as to avoid cross contamination; opening the reagent caps for a long time should also be avoided.
6. The final assay results are closely related to reagent effectiveness, the operations of analysts, and the test environment.
7. Our company is only responsible for the kits themselves, and will not be responsible for the sample consumption caused by kits during use. Users should fully consider the possible sample consumption before operation, and should reserve sufficient sample size.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com info@eaglebio.com

Jiangsu Hillgene Biopharma Co., Ltd.
Address: Building 4, Yuewang Wisdom Valley, 1463 Wuzhong Avenue, Wuzhong District, Suzhou, China
T.400 900 1882 W.www.hillgene.com