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CHO Residual DNA (qPCR) Detection Kit

CATALOG #DK-C004, 100 Tests

PRODUCT INTRODUCTION

The CHO Residual DNA (qPCR) Detection Kit enables specific, rapid quantification of residual CHO DNA in various biological products, such as interferons, interleukins, EPO, monoclonal antibodies, diagnostic reagents and recombinant protein vaccines. This kit can detect including CHO-K1, CHO-DXB11, CHO-S, and CHO-DG44 strains.

It is recommended to use KACTUS Host Cell Residual DNA (magnetic beads) Sample Preparation Kit (Catalog No. DK-SP001) for integrated workflow solutions.

KIT COMPONENTS

Components	Size	Storage conditions
CHO Primer & Probe Mix	550 µL × 1 vial	-20°C, protected from light
2X qPCR Reaction Mix	1.6 mL × 1 vial	-20°C
CHO DNA Quantitative Reference (30 ng/µL)	50 µL × 1 vial	
DNA Diluent	1.5 mL × 3 vials	

STORAGE AND SHELF LIFE

Unopened reagents maintain stability for 12 months from the date of manufacture when stored as recommended. After opening, please store the remaining reagents at -20°C, the reagents remain stable after 5 freeze-thaw cycles.

REQUIRED MATERIALS NOT SUPPLIED

- Real-time PCR instrument (ABI QuantStudio 3, ABI 7500, etc.), Centrifuge, Vortex
- Optical 8-tube strip or 96-well qPCR plate (sterile, nuclease-free, and low retention), 1.5mL centrifuge tube (sterile and low retention)

WORKFLOW

Prepare the reagents and samples

1. **Prepare the kit.** Place all components on ice to ensure complete dissolution before use.
2. Prepare DNA quantitative reference.
   a) Vortex the CHO DNA Quantitative Reference and DNA Diluent. Centrifuge briefly for 3-5 seconds.
   b) Dilute the CHO DNA Quantitative Reference (30 ng/µL) per the table below:

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Number	Final concentration (pg/μL)	Series dilution method	DNA Diluent
ST0	3000	5 μL CHO DNA Quantitative Reference （30 ng/μL）	45 μL
ST1	300	10 μL ST0	90 μL
ST2	30	10 μL ST1	90 μL
ST3	3	10 μL ST2	90 μL
ST4	0.3	10 μL ST3	90 μL
ST5	0.03	10 μL ST4	90 μL
ST6	0.003	10 μL ST5	90 μL

3. **Prepare the qPCR Working Mix:**
   a) Calculate the required number of qPCR reaction wells using the following formula:
      i. Total wells N = (1 Non-Template Control + References ST1 to ST6 + Test Samples) × 2 or 3 Number of Replicates.
   b) Prepare the Working Mix per the table below.
      i. Total Volume of Working Mix = 20 μL × (Total Wells N + 2)

Components	Volume Per Well
2X qPCR Reaction Mix	15 μL
CHO Primer & Probe Mix	5 μL
Total Volume	20 μL

4. **Prepare the qPCR reaction solution.** Place all the reagents on ice. Prepare the qPCR reaction solution per the below table.

	Sample	Reference ST1 to ST6	Non-Template Control (NTC)
Template	10 μL Sample	10 μL per ST	10 μL DNA Dilution
Working Mix per Well	20 μL	20 μL	20 μL
Total Volume per Well	30 μL	30 μL	30 μL

qPCR reaction parameters

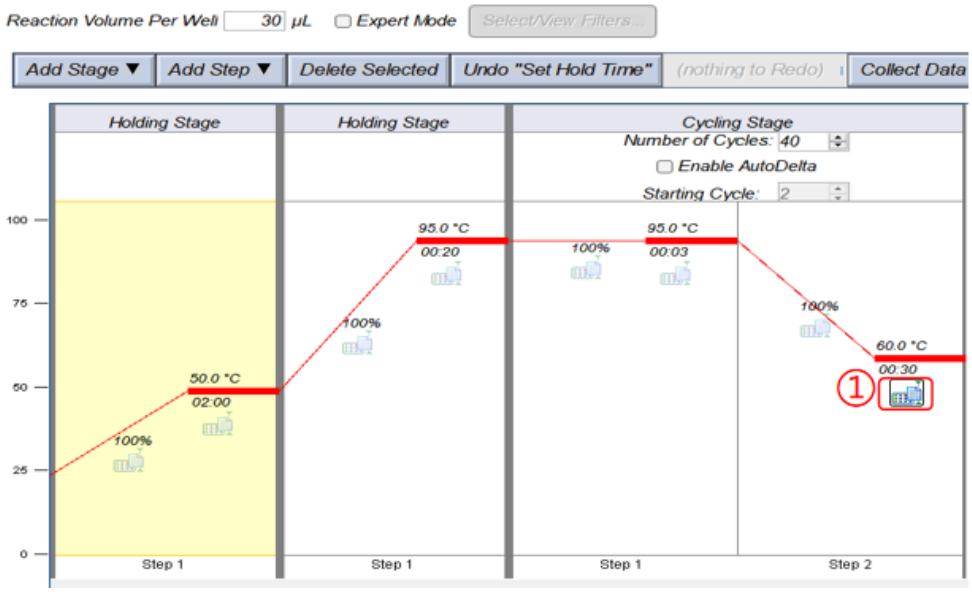
Using American ABI 7500 Real-Time PCR System (version 2.06) as an example:

1. Create a blank new program. Experiment type: Quantitation-Standard Curve. Reagents: TaqMan® Reagents.
2. Use “Add New Targets” to create new probes and edit the probe names in “Target Name”. Select the desired fluorescent and quenching groups in “Reporter” and “Quencher”. “Reporter” is selected as

FAM, the “Quencher” is selected as NONE, the fluorescent group of internal control (IC) is selected as VIC, and the reference fluorescence is ROX.

3. Use “Add New Samples” to add new samples and name them in the corresponding Sample Name column.
4. Set the reaction parameters. 60°C for 31 s in Stage 3 is set as fluorescence collection.

Stage 1	Digest	Reps: 1	50°C	2 min
Stage 2	Pre-denaturation	Reps: 1	95°C	20 s
Stage 3	Cycle	Reps: 40	95°C	3 s
			60°C	31 s



5. Click “Start Run” to perform qPCR.

ANALYSIS

Using American ABI 7500 Real-Time PCR System (version 2.06) as an example:

1. Click “Analyze”. Check whether the shape of the amplification curve is normal.
2. Read the results in the “View Well Table”page.

NOTES

1. The components in the kit are stable and do not require aliquoting.
2. Prepare negative and positive samples in separate areas avoiding cross-contamination.
3. Always use clean pipette tips and avoid contamination.
4. Use the kit before expiration and do not mix different lots.
5. Thaw all components completely before use. Mix well and centrifuge briefly.

6. Follow instructions carefully for the best results.
7. Results depend on reagent quality, technique, and lab conditions.
8. Prepare enough samples for your needs.
9. For research use only. Not for medical diagnosis.

FAQs

Common Issues	Possible Reasons	Actions to Take
No Ct value	Incorrect fluorescence detection settings in the PCR program	Ensure 60°C for 31 s in Stage 3 is set as fluorescence collection.
	Primer or probe degradation	Perform PAGE electrophoresis to determine if degradation had occurred.
	Template insufficient	Template degradation may be caused by impurities or damaged by repeated freeze-thaw.
Delayed Ct	qPCR reaction components degraded or insufficient	Verify the ROX reference signal.
	Inhibitors in template	
Negative control shows a signal	Contamination	1. Repeat using newly prepared Working Mix. 2. Prepare the reaction solution in the laminar flow hood. 3. Separate the different experiment areas. Change to low-retention, nuclease-free tubes and pipette tips.
Abnormal amplification curve	1. High concentration of template; template degraded 2. Evaporation or sealing issue 3. Bubbles in tube	1. Dilute template. Retest with fresh reagents. 2. Carefully transfer the reagents to avoid bubbles.

RELATED PRODUCTS

Product Name	Catalog No.
Host Cell Residual DNA (magnetic beads) Sample Preparation Kit	DK-SP001
CHO Residual DNA (qPCR) Detection Kit	DK-C004