Instruction Manual for GeticoSEQ CHO DNA Residue Quantitative Kit

1. Product Overview

The GeticoSEQ CHO DNA Residue Quantitative Kit is a professional detection tool designed for the **accurate quantification of residual host cell DNA from Chinese Hamster Ovary (CHO) cells** in biopharmaceutical products. Leveraging TaqMan fluorescent probe-based quantitative Polymerase Chain Reaction (qPCR) technology, the kit offers ultra-high sensitivity, strong specificity, and excellent reproducibility. It is widely applied in the quality control of biopharmaceuticals—including recombinant proteins, monoclonal antibodies, vaccines, and diagnostic reagents—covering the detection of intermediate samples, semi-finished products, and finished products. By enabling precise measurement of CHO DNA residues, the kit helps biopharmaceutical enterprises, research institutions, and third-party testing laboratories comply with global regulatory requirements (e.g., USP <509>/<1130>, Chinese Pharmacopoeia <3407>, ICH/WHO guidelines) and ensures the safety and quality of biopharmaceutical products.

2. Detection Principle

This kit adopts the **TaqMan fluorescent probe qPCR technology**, which combines the high specificity of probe hybridization with the high efficiency of PCR amplification. The core mechanism is as follows:

1. **Probe Design**: A TaqMan fluorescent probe specific to the conserved target sequence of CHO cell DNA is used. The 5'-end of the probe is labeled with a fluorescent reporter group (e.g., FAM), and the 3'-end with a quencher group (e.g., TAMRA). In the intact probe, the close proximity of the reporter and quencher groups causes the fluorescent signal to be quenched (no detectable fluorescence).
2. **PCR Amplification**: During the PCR cycle, primers first bind to the target DNA template and initiate extension under the action of hot-start Taq enzyme. When the enzyme extends to the probe-binding site, its 5'→3' exonuclease activity cleaves the probe into small fragments.
3. **Fluorescence Release**: Cleavage separates the reporter group from the quencher group, allowing the reporter group to emit a detectable fluorescent signal. The intensity of the fluorescent signal is directly proportional to the amount of amplified CHO DNA (i.e., the initial amount of residual CHO DNA in the sample).
4. **Quantification**: By monitoring the real-time fluorescent signal using a qPCR instrument, a standard curve is generated based on the cycle threshold (Ct value) of standard samples with known CHO DNA concentrations. The Ct value of the test sample is then compared to the standard curve to calculate the exact concentration of residual CHO DNA in the sample.

This technology effectively avoids non-specific amplification, with a minimum detection limit of the femtogram (fg) level, ensuring accurate quantification of trace CHO DNA residues.

3. Product Composition

All components of the kit are optimized for compatibility and performance. The detailed composition is shown in the table below:

Product Name	Specification	Detailed Description
2X qPCR Mix	12.5 μL / reaction	Contains optimized PCR buffer (adjusts ionic strength and pH for stable reactions), dNTPs (dATP, dCTP, dGTP, dTTP—raw materials for DNA synthesis), $MgCl_2$ (cofactor for Taq enzyme activity), and hot-start Taq enzyme (avoids low-temperature non-specific amplification).
CHO Primer & Probe Mix	2 μL / reaction	Includes primers and TaqMan fluorescent probes specifically designed for CHO cell DNA. Primers bind to conserved regions of CHO DNA to initiate amplification; probes recognize target sequences to ensure detection specificity (no cross-reaction with non-CHO DNA).
DNA Dilution Buffer	10 mL / bottle	Used for diluting CHO DNA Control (standard) and test samples. Maintains DNA stability (prevents degradation/aggregation) and ensures consistent reaction conditions across samples/standards, improving data reproducibility.
CHO DNA Control (10 ng/μL)	50 μL / tube	Standard reference material for preparing the standard curve. Calibrated against national standards, with high purity (no protein/ion interference) and stable concentration. Ensures traceability and accuracy of quantitative results.
RNase-free H_2O	10 mL / bottle	Used to make up the qPCR reaction volume. Treated to remove RNase contamination, avoiding interference with nucleic acid integrity and ensuring the purity of the reaction system.
50X ROX Reference Dye (Optional)	100 μL / tube	Used for calibrating fluorescent signals in qPCR instruments. Corrects well-to-well optical differences (e.g., uneven well

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		volume, light scattering) to improve detection accuracy. Added based on instrument requirements.

4. Storage and Stability

4.1 Storage Conditions

- Store the entire kit at **-20°C ± 5°C** in a constant-temperature freezer. Do not store in a frost-free freezer (repeated defrosting damages reagents).
- Sensitive components (e.g., CHO Primer & Probe Mix, 2X qPCR Mix) must be protected from light (wrap in aluminum foil) to prevent degradation of fluorescent groups or enzymes.
- Avoid repeated freeze-thaw cycles (≤3 cycles recommended). Upon first use, aliquot CHO Primer & Probe Mix and CHO DNA Control into small volumes (e.g., 10 μL/tube) to minimize freeze-thaw damage.

4.2 Shelf Life

- The kit is valid for **12 months from the date of manufacture** (see the product label for the exact expiration date) when stored as instructed.
- Before use, inspect all reagents for abnormalities:
  - Do not use 2X qPCR Mix if it shows precipitation, discoloration (normal color: pale yellow), or turbidity.
  - Discard CHO Primer & Probe Mix if it appears cloudy or has visible sediment.
  - Check CHO DNA Control for signs of degradation (e.g., abnormal viscosity); if present, replace with a new standard.

5. Sample Pretreatment

5.1 General Requirements

- Samples must be processed to remove interfering substances (e.g., high-concentration proteins, polysaccharides, detergents, nucleases) that may inhibit qPCR reactions.
- Use **RNase-free/DNase-free consumables** (e.g., 1.5 mL centrifugal tubes, pipette tips) and perform operations in a Class II biosafety cabinet or clean bench to avoid cross-contamination.
- For long-term storage, freeze processed samples at **-80°C** (storage at -20°C for >1 week may cause DNA degradation). Thaw samples on ice before use.

5.2 Pretreatment for Specific Sample Types

Different biopharmaceutical sample types require tailored pretreatment to ensure optimal detection results. Refer to the table below for detailed steps:

Sample Type	Pretreatment Steps

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Cell Culture Supernatant	1. Centrifuge at **10,000×g for 10 minutes** at 4°C to remove cell debris and insoluble impurities.2. Collect the supernatant; if the sample is highly concentrated, dilute it 1:1 to 1:10 with DNA Dilution Buffer (ensure the final concentration falls within the kit's detection range: 3 fg/μL–300 pg/μL).
Recombinant Protein/Monoclonal Antibody Bulk	1. Use a magnetic bead-based DNA purification kit (recommended: Getico Magnetic Bead Residual DNA Pretreatment Kit) to remove proteins: add 200 μL sample to 400 μL lysis buffer, incubate at 56°C for 15 minutes, then bind, wash, and elute DNA according to the purification kit instructions.2. Dilute the eluted DNA with DNA Dilution Buffer if needed.
Vaccine Intermediates	1. For inactivated vaccines: Add 100 μL sample to 300 μL proteinase K solution (20 mg/mL), incubate at 55°C for 30 minutes to digest proteins.2. Extract DNA using phenol-chloroform (or a commercial DNA extraction kit), then precipitate with ethanol and resuspend in 50 μL DNA Dilution Buffer.
Serum/Plasma Samples	1. Add protease inhibitor (final concentration: 1×) and nuclease inhibitor (final concentration: 1×) to the sample to prevent protein degradation and DNA cleavage.2. Dilute the sample 1:5 to 1:10 with DNA Dilution Buffer to reduce matrix interference, then centrifuge at 12,000×g for 15 minutes; use the supernatant for detection.

6. Operating Procedures

6.1 Preparation Work

1. **Reagent Equilibration**:
    - Remove CHO DNA Control and DNA Dilution Buffer from the freezer and thaw on ice.
    - Allow 2X qPCR Mix, CHO Primer & Probe Mix, and RNase-free H_2O to equilibrate to room temperature (20–25°C) for 30 minutes.
    - After thawing, gently invert all reagents 5–10 times to mix (avoid vigorous shaking, which may denature enzymes or generate bubbles).
    - Centrifuge all reagent tubes at **1,000–2,000 rpm for 10 seconds** to collect liquid at the bottom of the tube.
1. **Consumable and Instrument Preparation**:
    - Prepare clean RNase-free/DNase-free qPCR tubes or 96-well plates.
    - Calibrate pipettes (1 μL, 10 μL, 200 μL) to ensure accurate volume delivery.

- Preheat the fluorescent quantitative qPCR instrument and verify its status (e.g., thermal cycle accuracy, fluorescence detection sensitivity).

6.2 Preparation of Standard Curve

The kit uses CHO DNA Control (10 ng/μL) to prepare a 7-point standard curve (concentrations: 300 pg/μL, 30 pg/μL, 3 pg/μL, 300 fg/μL, 30 fg/μL, 3 fg/μL, and 0 fg/μL (blank)). Follow these steps:

1. Label 7 clean 1.5 mL centrifuge tubes as **STD 0 (blank)**, **STD 1**, **STD 2**, **STD 3**, **STD 4**, **STD 5**, and **STD 6**.
2. Add 90 μL DNA Dilution Buffer to each tube (STD 0 to STD 6).
3. Add 10 μL CHO DNA Control (10 ng/μL) to STD 6; gently pipette 15–20 times to mix. After short centrifugation (1,000 rpm for 10 seconds), the concentration of STD 6 is **300 pg/μL**.
4. Perform serial 10-fold dilutions: Transfer 10 μL from STD 6 to STD 5, mix thoroughly, and centrifuge briefly—STD 5 concentration = **30 pg/μL**. Repeat this step for STD 4 (3 pg/μL), STD 3 (300 fg/μL), STD 2 (30 fg/μL), and STD 1 (3 fg/μL). STD 0 remains as DNA Dilution Buffer (0 fg/μL, blank control).
5. **Note**: Use a new pipette tip for each dilution to avoid cross-contamination. Prepare the standard curve fresh before each experiment; do not reuse diluted standards.

6.3 Preparation of Controls

To ensure the validity of the experiment, three types of controls must be set up:

Control Type	Purpose	Preparation Steps
External Reference Control (ERC)	Verify qPCR reaction efficiency and sample matrix interference	1. Add 100 μL processed test sample to a clean tube.2. Add 10 μL STD 3 (300 fg/μL CHO DNA Control) and mix thoroughly.3. Treat the ERC sample with the same DNA extraction/purification steps as the test sample (if applicable).
Negative Control Sample (NCS)	Detect external contamination (e.g., sample cross-contamination)	1. Add 100 μL sample matrix (e.g., DNA Dilution Buffer, blank culture medium) to a clean tube.2. Process the NCS sample with the same steps as the test sample (extraction/purification, if applicable).
No Template Control (NTC)	Detect reagent or consumable contamination	Prepare a 20 μL qPCR reaction system without DNA template: 12.5 μL 2X qPCR Mix + 2 μL CHO Primer & Probe Mix + 0.4 μL 50X ROX Reference Dye (if used) + 5.1 μL RNase-free H_2O.

6.4 Preparation of qPCR Reaction System

Prepare the reaction system on ice to prevent enzyme activation. The recommended system volume is 20 µL per well/tube. Prepare a **master mix** for multiple reactions first (add 5% extra volume to compensate for pipetting loss), then distribute to individual wells/tubes before adding the template.

Component	Volume per Reaction (20 µL)	Notes
2X qPCR Mix	12.5 µL	Do not freeze-thaw more than 3 times; mix gently before use.
CHO Primer & Probe Mix	2 µL	Protect from light; avoid prolonged exposure to room temperature.
50X ROX Reference Dye (Optional)	0.4 µL	Add only if required by the qPCR instrument (final concentration = 1X). If not used, replace with 0.4 µL RNase-free H_2O.
RNase-free H_2O	0.1 µL	Adjust volume based on whether ROX is added (e.g., 0.5 µL if ROX is not used).
DNA Template	5 µL	Add 5 µL of each standard (STD 0–STD 6), test sample, ERC, NCS, or NTC (NTC uses 5 µL RNase-free H_2O instead of template).

Steps to prepare the reaction system:

1. Calculate the total volume of master mix needed (e.g., for 20 reactions: 20 × 1.05 = 21 reactions).
2. Mix 2X qPCR Mix, CHO Primer & Probe Mix, ROX Reference Dye (if used), and RNase-free H_2O in a clean tube; gently pipette 3–5 times to mix.
3. Dispense 15 µL of master mix into each well/tube of the qPCR plate/tube.
4. Add 5 µL of template (standard, sample, ERC, NCS, or NTC) to the corresponding wells/tubes.
5. Seal the plate/tube with a qPCR-compatible sealing film; centrifuge at **1,000–2,000 rpm for 10 seconds** to remove bubbles and ensure the reaction mixture is at the bottom.

6.5 qPCR Amplification and Detection

1. Load the sealed qPCR plate/tube into the fluorescent quantitative qPCR instrument.
2. Set the instrument parameters according to the following recommendations (adjust based on instrument model if needed):

Stage	Temperature	Time	Cycles	Fluorescence Collection
Pre-denaturation	95°C	3–5 minutes	1	No

Denaturation	95°C	15–30 seconds	40–45	No
Annealing & Extension	60°C	30–60 seconds	40–45	Yes (collect FAM signal)

1. Select the fluorescent channel: Set the reporter dye to **FAM** and the quencher dye to **TAMRA** (or “None” if the instrument defaults to passive reference).
2. Start the qPCR run. After the run is complete, save the raw data (Ct values and amplification curves) for analysis.

6.6 Result Analysis

6.6.1 Validation of Experimental Validity

Before analyzing sample results, confirm that the following criteria are met (otherwise, the experiment is invalid and must be repeated):

1. **Standard Curve**: The linear correlation coefficient (R^2) ≥ 0.99; the slope is between -3.6 and -3.1 (corresponding to a qPCR efficiency of 90%–110%).
2. **ERC**: The recovery rate of CHO DNA is 80%–120% (recovery rate = (Detected concentration of ERC - Detected concentration of test sample) / Spiked concentration × 100%).
3. **NCS/NTC**: No amplification (Ct value > 45 or undetermined); if amplification occurs, there is contamination, and the experiment must be repeated.

6.6.2 Calculation of Residual CHO DNA

1. **Standard Curve Equation**: Use the qPCR instrument software to generate a standard curve with “Ct value” as the Y-axis and “log10 (CHO DNA concentration, fg/μL)” as the X-axis. Obtain the regression equation: **Y = aX + b** (a = slope, b = intercept).
2. **Sample Concentration Calculation**: Substitute the sample’s Ct value into the regression equation to calculate the log10 (concentration) of CHO DNA in the sample. Convert to the actual concentration (C_sample, fg/μL) using 10^(X).
3. **Original Sample Residual Calculation**: Adjust for sample dilution and pretreatment steps using the formula:

Residual CHO DNA (fg/mL or fg/mg) = C_sample × Dilution factor × Elution volume / Sample volume

- *Example*: If a 100 μL protein sample is purified and eluted in 50 μL DNA Dilution Buffer (dilution factor = 0.5), and C_sample = 30 fg/μL, then residual CHO DNA = 30 fg/μL × 0.5 × 50 μL / 0.1 mL = 7,500 fg/mL = 7.5 pg/mL.

6.6.3 Result Judgment

Compare the calculated residual CHO DNA with the applicable regulatory limits (e.g., WHO/ICH recommends ≤10 ng CHO DNA per dose for biopharmaceuticals):

- If the residual amount is **below the limit**, the sample meets quality requirements.
- If the residual amount is **above the limit**, investigate potential causes (e.g., incomplete purification, sample contamination) and re-test after optimizing the process.

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7. Precautions

1. **Contamination Prevention**:
    - Designate separate work areas for sample pretreatment, reagent preparation, and qPCR setup to avoid cross-contamination.
    - Use disposable RNase-free/DNase-free consumables; do not reuse pipette tips or tubes.
    - Clean the workbench, pipettes, and instrument with a nuclease remover (e.g., DNAzap) before and after the experiment.
    - Dispose of used reagents and consumables as **biohazardous waste** (e.g., autoclave at 121°C for 30 minutes before disposal).
1. **Reagent Handling**:
    - Do not use reagents beyond the expiration date or with visible abnormalities (precipitation, discoloration).
    - Thaw CHO DNA Control and DNA Dilution Buffer on ice; other reagents can be thawed at room temperature, but avoid prolonged exposure.
    - After use, immediately return reagents to -20°C storage (except for RNase-free H_2O, which can be stored at 4°C for short-term use).
1. **Sample Handling**:
    - Ensure sample pretreatment is complete (e.g., no residual proteins/nucleases) to avoid inhibiting qPCR reactions.
    - If the sample concentration is outside the kit's detection range, adjust the dilution factor (do not dilute beyond 1:1000, as this may reduce accuracy).
1. **Instrument Maintenance**:
    - Calibrate the qPCR instrument every 6 months (or as recommended by the manufacturer) to ensure thermal cycle accuracy and fluorescence detection sensitivity.
    - Clean the instrument's optical module regularly to remove dust or residual fluorescence, which may interfere with signal collection.
1. **Troubleshooting Common Issues**:

Issue	Possible Cause	Solution
Low qPCR efficiency (slope < -3.6)	Degraded Taq enzyme; incorrect primer/probe concentration	Replace 2X qPCR Mix or CHO Primer & Probe Mix; verify reagent volumes.
NTC shows amplification	Reagent/consumable contamination; aerosol carryover	Use new reagent aliquots and consumables; clean the workbench with a nuclease remover; use a pipette with a filter tip.
Sample Ct value > 45 (undetectable)	Sample dilution too high; DNA degradation	Reduce the dilution factor; re-extract DNA from the original sample (store at -80°C to prevent degradation).

Poor standard curve linearity ($R^2 < 0.99$)	Inaccurate standard dilution; uneven mixing	Re-prepare the standard curve with precise pipetting; ensure thorough mixing of each dilution step.

8. Contact Information

For technical support, product inquiries, or complaints, please contact:

- **Manufacturer**: Getico Scientific Co., Ltd.
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