GeticoSEQ 293 DNA Residue Quantitative Kit Instruction Manual

1. Product Overview

The GeticoSEQ 293 DNA Residue Quantitative Kit is elaborately developed for the accurate determination of residual host cell DNA from human embryonic kidney 293 (HEK293) series cells (such as HEK293, 293T, etc.) in biological products. It boasts extremely high sensitivity and specificity, and is widely applied in the field of biological products. It can be used for the detection of intermediate samples, semi-finished products and finished products of various biological products, including adeno-associated virus/lentivirus-related products for gene and cell therapy, stem cell products and protein products. This kit provides key data support for quality control and safety evaluation of biological products.

During the production of biological products, especially in the field of gene and cell therapy, a variety of viral vectors are often produced using 293 series cells. However, during the product purification stage, a small amount of HEK293 cell DNA may remain. This residual DNA may trigger immune responses or carry unknown gene fragments, posing potential risks. Therefore, accurate detection of its content is of great significance. This kit has undergone rigorous research, development and verification, and can meet the strict standards for HEK293 DNA residue detection set by biological product manufacturers, quality control laboratories and regulatory authorities.

2. Detection Principle

This kit adopts TaqMan fluorescent probe combined with polymerase chain reaction (PCR) technology. During the PCR amplification process, the TaqMan fluorescent probe specifically binds to the target sequence of HEK293 cell DNA. The 5' end of the probe is connected to a fluorescent reporter group (such as FAM), and the 3' end is connected to a quencher group (such as TAMRA). In the intact probe structure, due to the close distance between the fluorescent reporter group and the quencher group, the fluorescent signal is quenched, and no obvious fluorescence can be detected.

When the primer extends to the probe binding site, the 5'-3' exonuclease activity of Taq enzyme cleaves and degrades the probe, separating the fluorescent reporter group at the 5' end of the probe from the quencher group at the 3' end. At this time, the fluorescent reporter group emits fluorescence. With the increase of PCR cycles, the amount of amplified DNA increases exponentially, and the corresponding fluorescent signal intensity also increases proportionally. By real-time monitoring the change of fluorescent signal with a real-time fluorescent quantitative PCR instrument, and comparing the fluorescent signal of the sample with that of the standard product with known concentration, the residual amount of HEK293 cell DNA can be accurately quantified. This technology can effectively avoid the interference of non-specific amplification, ensuring the reliability of detection results. The minimum detection limit can reach the fg level, which meets the requirement of trace HEK293 DNA residue detection. Meanwhile, the unique primer design of this kit ensures the detection capability of fragmented DNA, further improving the comprehensiveness and accuracy of detection.

3. Product Composition

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Product Name	Specification	Detailed Description
2X qPCR Mix	12.5 μl / reaction	It contains an optimized PCR buffer, which accurately adjusts the ionic strength and pH value of the reaction system to create a stable environment for the PCR reaction; dNTPs (dATP, dCTP, dGTP, dTTP) serve as raw materials for DNA synthesis, and are supplied in sufficient quantities to ensure the smooth progress of amplification; $MgCl_2$ provides essential cofactors for the activity of Taq enzyme, and its concentration is optimized to balance the amplification efficiency and specificity; the hot-start Taq enzyme is inactive at room temperature and needs to be activated at high temperature, which effectively avoids low-temperature non-specific amplification and improves the specificity and accuracy of amplification.
293 Primer & Probe Mix	2 μl / reaction	Primers specifically designed for HEK293 cell DNA. Their sequences have been analyzed by bioinformatics and verified by experiments, enabling them to accurately bind to specific target regions of HEK293 cell DNA and initiate efficient amplification; the supporting TaqMan fluorescent probe also specifically recognizes the target sequence, ensuring high-specificity amplification and detection of HEK293 DNA, and reducing cross-reaction with DNA of other species.
DNA Dilution Buffer	/	It is used to dilute DNA standards and samples. Its components are optimized to maintain the stability of DNA and reduce DNA degradation or aggregation during dilution. At the same time, it ensures the consistency of the reaction system after dilution of different samples and standards, improves the repeatability and reproducibility of the experiment, and ensures reliable results.
293 DNA Control (10 ng/μl)	/	It is used as a standard to prepare the standard curve. Its preparation follows strict standard operating procedures, is calibrated by national standard samples, has high purity, and has undergone multiple tests to ensure no protein and ion interference. The high-purity standard provides a reliable

		reference for the accuracy of quantitative detection, and helps to accurately determine the residual amount of HEK293 DNA in samples.
RNase-free H_2O	/	It is used to prepare the reaction system and has been specially treated to be free of RNase contamination. In operations involving RNA extraction (if any), it can avoid potential impacts on RNA samples, maintain the purity of the reaction system, and ensure that the experimental results are not interfered by RNase.
50X ROX Reference Dye (optional)	/	It is used to calibrate the fluorescent signal. There may be slight optical differences between different PCR reaction wells. The ROX dye generates a stable fluorescent signal to calibrate these differences, eliminates the difference in fluorescent background between wells, and improves the accuracy of detection results. It can be added according to the needs of the fluorescent quantitative PCR instrument. If the instrument itself has other calibration methods or no additional calibration is required, it may not be used.

4. Scope of Application

1. Monitoring of biological product production process: In the production of lentiviruses and adeno-associated viruses for gene and cell therapy, the detection of culture supernatants after virus transfection of 293 series cells can timely detect cell DNA leakage; the detection of samples after each step of the purification process can evaluate the effect of the purification process on HEK293 DNA removal, thereby optimizing the purification process; in the production of stem cell and protein products, the residual amount of HEK293 DNA is monitored in each link from cell culture to product purification to ensure that the product quality meets the requirements.
2. Quality control and release testing: It is used by biological product quality control laboratories to detect the residual amount of HEK293 DNA in batch products. Based on the pre-set quality standards, it is judged whether the product is qualified and whether it can be released to the market. Strict quality control helps enterprises ensure stable and consistent product quality, enhance corporate reputation, and reduce the risk of product recalls caused by product quality problems.
3. Regulatory compliance testing: It meets the strict regulatory requirements of national drug regulatory authorities (such as FDA, EMA, NMPA, etc.) for residual host cell DNA in biological products. For example, the World Health Organization (WHO) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines recommend that the residual host cell DNA in biological products should not exceed 10 ng per dose. Through detection with this kit, enterprises can accurately grasp the residual status of

HEK293 DNA in products, helping to ensure product compliance and smoothly pass inspections and approvals by regulatory authorities.

5. Operating Procedures

(I) Preparation Work

1. Take out all reagents of the kit from the refrigerator. Thaw the DNA Control and DNA Dilution Buffer on ice first to prevent rapid temperature changes from affecting the stability of the reagents. Let other reagents equilibrate slowly at room temperature for about 30 minutes to make the temperature of the reagents evenly close to room temperature and reduce experimental errors caused by temperature differences. After the reagents are completely thawed, gently invert and mix them, avoiding violent shaking to generate bubbles, as violent shaking may damage the enzyme structure or cause aggregation of primers, probes and other molecules. Then centrifuge at low speed for a short time (about 1000-2000 rpm for 10 seconds) to concentrate the reagents at the bottom of the tube for accurate aspiration.
2. Prepare clean RNase-free PCR reaction tubes or 96-well plates, pipettes and matching tips. Calibrate the pipette before use to ensure accurate pipetting volume. Use newly opened tips during operation to prevent cross-contamination. Wipe and disinfect the experimental workbench with 75% alcohol first, then clean it with a nuclease remover to create a clean experimental environment and avoid interference of nucleases in the environment with the experiment.
3. Prepare the samples to be tested as needed. The samples should be solutions that have undergone appropriate treatment (such as nucleic acid extraction, purification, etc.) to ensure that the DNA in them can be amplified. If the sample is a biological product stock solution, dilute it appropriately according to the actual situation so that the DNA concentration in the final reaction system is within the detection range of the kit. The appropriate dilution factor can be determined through pre-experiments or by referring to the detection experience of similar samples. For samples with complex components, such as those containing high concentrations of proteins and polysaccharides, it may be necessary to optimize the extraction and purification steps to remove interfering substances and improve detection accuracy.

(II) Preparation of Standard Curve

1. Take 7 clean 1.5 ml centrifuge tubes and clearly mark them as STD 0, STD 1, STD 2, STD 3, STD 4, STD 5, and STD 6 respectively to prevent confusion.
2. In the STD 0 centrifuge tube, add 90 μl of DNA Dilution Buffer and 10 μl of 293 DNA Control. Gently pipette and mix 15-20 times with a pipette to avoid generating bubbles and ensure uniform mixing. After short-term centrifugation (1000-2000 rpm for 10 seconds), the DNA concentration is 3 ng/μl at this time. Aliquot and store the diluted DNA standard. It can be stored short-term (no more than 3 months) at -25°C to -15°C, and repeated freezing and thawing should be avoided, as repeated freezing and thawing can easily lead to DNA degradation and affect the accuracy of the standard curve.
3. Add 90 μl of DNA Dilution Buffer to each of the other 6 labeled centrifuge tubes. Aspirate 10 μl of the solution from the STD 0 tube and add it to the STD 1 tube. Mix thoroughly by gently inverting the centrifuge tube 10-15 times or slowly pipetting with a pipette for 15 seconds to 1 minute to ensure uniform concentration. After short-term centrifugation, the DNA concentration in the STD 1 tube is 300 pg/μl. Use this method to perform serial dilution on the subsequent centrifuge tubes in turn to obtain DNA standards with different concentration gradients, with concentrations of 300

pg/μl, 30 pg/μl, 3 pg/μl, 300 fg/μl, 30 fg/μl, and 3 fg/μl in sequence. Set 3 replicate wells for each concentration of the standard. Repeated experiments can improve the reliability and accuracy of the data and reduce experimental errors.

(III) Sample Preparation

1. Set up the External Reference Control (ERC): Determine the concentration of HEK293 DNA in the ERC according to the experimental requirements (taking 30 pg of HEK293 DNA as an example). In a clean 1.5 ml centrifuge tube, add 100 μl of the sample to be tested, then add 10 μl of 293 DNA standard (STD 3) with a concentration of 3 pg/μl. Mix thoroughly by vortexing for 10-15 seconds or repeatedly pipetting with a pipette, and mark it as ERC. Then perform DNA extraction together with the sample to be tested (if the sample has completed the extraction step, proceed directly to the subsequent operation) to prepare the purified ERC sample. The positive control is used to verify the effectiveness of the entire detection process and ensure that the experimental conditions can accurately detect the known amount of HEK293 DNA.
2. Set up the Negative Control Sample (NCS): Add 100 μl of sample matrix (if there is no special instruction, it is generally DNA Dilution Buffer) to a clean 1.5 ml centrifuge tube and mark it as NCS. Perform DNA extraction on the NCS sample together with the sample to be tested (if the sample has completed the extraction step, proceed directly to the subsequent operation) to prepare the purified NCS sample. The negative control is used to detect whether there is external contamination during the experiment. If the negative control shows a positive result, it indicates that there is contamination in the experimental process, and the cause needs to be investigated and the experiment should be repeated.
3. Set up the No Template Control (NTC): For each tube or well of NTC reaction, prepare a 20 μl reaction mixture, which is 15 μl of 293 qPCR Mix + 4 μl of 293 Primer & Probe Mix + 1 μl (if there is internal reference-related reagent, add the internal reference reagent; if not, add RNase-free H_2O) + 10 μl of DNA Dilution Buffer. It is recommended to set 3 replicate wells. The no-template control is used to detect whether there is non-specific amplification in the PCR reaction system. If the NTC shows an amplification curve, it indicates that there is contamination in the reaction system or non-specific binding of primers and probes. It is necessary to optimize the reaction system or re-prepare the reagents.

(IV) Preparation of Reaction System (Taking 20 μl System as an Example)

In an RNase-free PCR reaction tube or 96-well plate on ice, add each component in the following order:

1. DNA template: Add 5 μl of standard solutions with different concentration gradients for the standard, aspirate accurately, and avoid the pipette tip touching the wall of the reaction tube to prevent cross-contamination; add 5 μl of the treated and diluted sample solution to be tested for the sample to ensure uniform aspiration; add 5 μl of the corresponding sample solution for ERC, NCS and NTC respectively.
2. 2X qPCR Mix: Add 12.5 μl to provide various components required for the PCR reaction. Add it slowly along the tube wall to avoid generating bubbles.
3. 293 Primer & Probe Mix: Add 2 μl to initiate the specific amplification and detection of HEK293 DNA. Insert the pipette tip into the bottom of the reagent tube to aspirate to ensure accurate aspiration volume.

4. If 50X ROX Reference Dye is used, add it in an appropriate proportion according to the instrument requirements (for example, if the instrument requires a final ROX concentration of 1X, add 0.4 μl of 50X ROX Reference Dye). If the instrument does not require ROX calibration, make up the volume with RNase-free H_2O. When adding the ROX dye, pay attention to fully mixing it with other reagents.
5. RNase-free H_2O: Make up the volume to 20 μl for the reaction. Add it drop by drop to avoid volume error.

After adding each component, gently pipette and mix with a pipette 3-5 times to avoid generating bubbles. Then centrifuge the reaction tube or 96-well plate at low speed for a short time (1000-2000 rpm for 10 seconds) to concentrate the reaction solution at the bottom of the tube or well, ensuring the uniformity of the reaction system.

(V) PCR Amplification and Detection

1. Place the prepared reaction tube or 96-well plate into the fluorescent quantitative PCR instrument, and perform PCR amplification according to the following recommended procedures (different models of PCR instruments may need to be adjusted appropriately according to their characteristics):

- Pre-denaturation: 95°C for 3-5 minutes to activate the hot-start Taq enzyme, fully denature the DNA template, and open the double-stranded structure to prepare for subsequent primer binding and amplification.
- Cyclic reaction (40-45 cycles): 95°C for 15-30 seconds to denature the DNA double strand, break the hydrogen bond, and form a single-stranded template; 60°C for 30-60 seconds for primer annealing and extension. At this temperature, the primer specifically binds to the single-stranded template, and the Taq enzyme uses the primer as the starting point and dNTPs to synthesize a new DNA strand. At the same time, the fluorescent signal is collected. The fluorescent signal is stable and highly specific at this temperature.

1. After the amplification is completed, the instrument automatically generates a curve (Ct value) showing the change of the fluorescent signal with the number of cycles. According to the relationship between the Ct value of the standard curve and the corresponding DNA concentration, the concentration of HEK293 DNA in the sample is obtained through the instrument software or manual calculation. The linear correlation coefficient (R^2) of the standard curve should be greater than 0.99 to ensure the reliability of the standard curve. If the R^2 value does not meet the requirements, it is necessary to re-prepare the standard curve and conduct the experiment. The reasons for the poor R^2 value may include inaccurate dilution of the standard, contamination of the reaction system, instrument failure, etc., which need to be investigated one by one.

(VI) Result Analysis

1. Calculate the residual amount of HEK293 DNA in the sample: Substitute the Ct value of the sample into the standard curve equation to calculate the concentration of HEK293 DNA in the sample (unit: fg/μl or pg/μl, etc.). Then, combined with the dilution factor of the sample, sampling volume and other information, calculate the residual amount of HEK293 DNA in the original sample (such as the content of HEK293 DNA per milligram of protein, the content of HEK293 DNA per milliliter of biological product, etc.). Carefully check the data during the calculation process to avoid errors.

2. Result judgment: Compare the calculated residual amount of HEK293 DNA in the sample with the relevant quality standards or regulatory requirements. If the residual amount of HEK293 DNA in the sample is lower than the specified limit, the sample meets the quality requirements; if it is higher than the limit, it is necessary to further analyze the reasons, such as whether there is a problem in the production process, whether the sample processing process introduces contamination, etc., and take corresponding improvement measures. For example, if a problem in the production process is suspected, each link of the production process can be investigated to optimize the purification process; if contamination in sample processing is suspected, the sample processing process needs to be re-evaluated and the experimental operation specifications should be strengthened.

6. Precautions

1. Reagent Storage and Stability: The kit should be stored at -20°C ± 5°C, and repeated freezing and thawing should be avoided. Repeated freezing and thawing may lead to a decrease in enzyme activity, degradation of primers and probes, etc., which will affect the experimental results. Centrifuge the reagent tube briefly before use to ensure that the reagent is concentrated at the bottom of the tube. The kit is valid for [X] months from the date of production under the specified storage conditions (see the product label for the specific validity period). The performance of the kit beyond the validity period cannot be guaranteed, so it should not be used. Check the appearance of the reagent before use. If there are abnormalities such as precipitation and discoloration, stop using it.
2. Contamination Prevention: The entire operation process should be carried out in a clean environment. Strictly use RNase-free consumables, and wear gloves and masks during operation to prevent cross-contamination. Clean the experimental workbench with a nuclease remover regularly. Dispose of the used waste reagents and consumables in accordance with the regulations for biological hazardous substances to prevent environmental pollution. For example, put the waste PCR reaction tubes and tips into a special biological hazardous waste collection container, and conduct unified autoclaving and other treatments.
3. Primers and Probes: The 293 Primer & Probe Mix is sensitive to temperature, so minimize its exposure time at room temperature during use. Take it out of the refrigerator and use it immediately, and put it back as soon as possible. If any abnormality such as precipitation or discoloration is found in the primers or probes, do not use them, as this may affect the accuracy of the experimental results. Precipitation may be the aggregation of primers or probes, and discoloration may indicate structural changes, both of which will affect their binding ability to the target DNA.
4. Sample Processing: Ensure that the DNA is not degraded or lost during the sample processing process. For samples with complex components (such as high concentrations of proteins, polysaccharides, nuclease inhibitors, etc.), additional purification steps may be required to remove interfering substances and ensure the smooth progress of the PCR reaction. If the sample processing method is not certain, it is recommended to conduct a pre-experiment to optimize the sample processing conditions. For example, try different nucleic acid extraction methods or purification columns, and compare their effects on the extraction of HEK293 DNA from the sample and the subsequent PCR reaction.
5. Instrument Calibration: Before using the fluorescent quantitative PCR instrument, ensure that the instrument has been calibrated and its performance is normal. Regularly check the key components of the instrument, such as the fluorescence detection system and the thermal cycle system, to ensure the accuracy of the experimental data.