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# Instructions for Use of Residual K562 feeder cell Detection Kit

**(Cat.No. HG-KF001)**

This kit is intended for research use only and is not intended for diagnosis.

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## 1. Product Introduction

K562 cells engineered via gene editing are widely studied and utilized for their excellent proliferative properties. Research has shown that using gene-edited K562 cells as feeder cells can enhance NK cell expansion efficiency by tens to tens of thousands of times. Recently, the FDA's guideline document "Considerations for the Use of Human- and Animal-Derived Materials in the Manufacture of Cellular and Gene Therapy and Tissue-Engineered Medical Products" has mentioned that irradiated K562 cells can be used as feeder cells for in vitro culture.

Although K562 cells are irradiated and easily eliminated by NK cells, it is essential to ensure that residual feeder cells are minimized in the final product to ensure patient safety. Researchers are advised to develop highly sensitive detection methods, such as reverse transcription-polymerase chain reaction (RT-PCR) or droplet digital PCR (ddPCR), and validate these methods for specificity, accuracy, precision, and detection limits to effectively characterize the manufacturing process and final product, minimizing impacts on product quality, clinical efficacy, and safety.

This kit utilizes RT-PCR with specifically designed target sites for the detection of residual K562 feeder cells.

## 2. Detection Range

10 copies/μL ~ $1\times10^6$ copies/μL, with a low detection limit for K562 cells of 0.05%.

## 3. Applications

Detection of residual K562 feeder cells in cellular preparation samples.

## 4. Kit Specifications

100 Test

## 5. Kit Contents

| Contents | Specifications | Storage Conditions |
|---|---|---|
| K562 Quantitative Standard（$1\times10^8$ copies/μL） | 50μL × 1 | ⩽ -18°C |
| K652 Primer&Probe MIX | 550μL × 1 | ⩽ -18°C， Protect from Light |
| 2×qPCR Reaction Buffer | 1.2mL × 1 | ⩽ -18°C，Protect from Light |
| DNA Diluent | 1.5mL × 3 | ⩽ -18°C |
| DNase/RNase-Free Water | 1.0mL × 1 | ⩽ -18°C |
| 50× ROX High | 50μL× 1 | ⩽ -18°C，Protect from Light |
| 50× ROX Low | 50μL× 1 | ⩽ -18°C，Protect from Light |

## 6. Expiration Date

Store the kit components under the specified storage conditions,.valid for 18 months. Refer to the expiration date on the

outer packaging for the shelf life.I

## 7. Compatible Instruments (including but not limited to)

- ABI PRISM 7500
- FQD-96A(Bioer)
- CFX96(Bio-Rad)
- Roche Light Cycler 480

| Instruments | ROX Reference Dye |
|---|---|
| ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne, StepOne Plus | 50× ROX High |
| ABI 7500, 7500 Fast, ViiA7, QuantStudio 3 and 5, QuantStudio 6,7,12k Flex. Stratagene MX3000P, MX3005P, MX4000P | 50× ROX Low |
| Bio-Rad CFX96, CFX384, iCycler iQ, iQ 5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4.Roche Applied Science LightCycler 480, LightCycler 2.0; Lightcycler 96.Eppendorf Mastercycler ep realplex, realplex 2 s. Qiagen Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000. Thermo Scientific PikoReal Cycler.Cepheid SmartCycler.Illumina Eco qPCR | No ROX |

## 8. Requirement of Instrument, Equipment & Consumable

(1) Fluorescent quantitative PCR instrument (with FAM and VIC channels).

(2) Vortex mixer.

(3) Mini centrifuge.

(4) RNA extraction reagents.

(5) Reverse transcription reagents.

(6) Pipettes and tips.

(7) PCR Eight-strip tubes with attached caps or 96-well PCR half skirt reaction (lates with sealing film).

## 9. Safety Precautions

For your safety and health, wear a lab coat, mask, gloves, and proper eye and face protection during operation to avoid contact between reagents and skin or eyes.

## 10. Experimental Steps

10.1 RNA Extraction

Extract RNA from a specific volume of cell suspension (1E6 ~ 5E6 cells).

10.2 Reverse Transcription

Perform genomic DNA removal and reverse transcription on the extracted RNA to obtain cDNA for detection.

10.3 PCR Amplification

10.3.1 Preparation of Standard Solutions:

Thaw reference standards and DNA diluent at 4°C. Once thawed, vertex by inverting 20 times and centrifuge at 6000 rpm for 5 seconds. Dilute the RNA reference standard (1E+08 copies/μL) with DNA diluent in a gradient to 1E+06, 1E+05, 1E+04, 1E+03, 1E+02, and 10 copies/μL. Perform serial dilutions as table below (The standard sample is diluted by pipetting and mixing, and pipetting and mixing is repeated 20 times):

| Sample Name | Volume of Diluent and Sample | Concentration（copies/μL） |
|---|---|---|
| STD0 | 10μL Standard + 90μL DNA Diluent | 1E+07 |
| STD1 | 10μLSTD0 + 90μL DNA Diluent | 1E+06 |
| STD2 | 10μL STD1 + 90μL DNA Diluent | 1E+05 |
| STD3 | 10μL STD2 + 90μL DNA Diluent | 1E+04 |
| STD4 | 10μL STD3 + 90μL DNA Diluent | 1E+03 |
| STD5 | 10μL STD4 + 90μL DNA Diluent | 1E+02 |
| STD6 | 10μL STD5 + 90μL DNA Diluent | 1E+01 |

10.3.2 PCR Reaction Mix Preparation:

Calculate the required number of reaction wells based on the number of samples, with three replicates for each sample. Note: Add two extra wells to the total for loss. Prepare the PCR mix in a biosafety cabinet as follows:

| Content Name | Usage（μL） |
|---|---|
| 2× qPCR Reaction Buffer | 10 |
| K562 Primer&Probe MIX | 4.6 |
| 50× ROX | 0.4 |

Note: If the instrument does not need to add ROX, 50× ROX can be replaced with DNase/RNase-Free Water.

The sample loading conditions of each reaction well are shown in the table:

| Sample Name | Reaction Volume |
|---|---|
| Standard Curve | 15μL PCR MIX + 5μL(STD1/STD2/STD3/STD4/STD5/STD6) |
| Blank Control | 15μL PCR MIX + 5μL DNA Diluent |
| Test Sample | 15μL PCR MIX + 5μLTest Sample / Extraction Recovery Control RNA Extracte |

10.3.3 Sample Loading:

After loading, cap the eight-strip tubes by first securing the ends and then the middle. Mark the caps for reference.

10.3.4 Centrifugation and Mixing:

After sealing the tubes, centrifuge at 6000 rpm for 10 seconds to collect liquid at the bottom. Vortex for at least 10 seconds at speed 6 to mix thoroughly, and centrifuge again at 6000 rpm for 10 seconds to collect liquid. Remove any bubbles.

10.3.5 PCR Program Setup:

Open the PCR instrument and set up the PCR reaction program follow the below table.

| Program | Temperature | Time | Cycle Times | Note |
|---|---|---|---|---|
| Pre- Denaturation | 95°C | 3 minutes | 1 | NA |
| Denaturation | 95°C | 15 Seconds | 45 | NA |
| Annealing & Extension | 60°C | 60 Seconds | | Fluorescence Signal Acquisition |

10.3.6 Sample Plate Setup: Configure the sample plate by editing information for standards, NTC, negative control, ERC, and Test Samples. Assign fluorescence channels (FAM for K562, VIC for ABL) and input replicate wells and sample names. For reference standards(STD1, STD2, STD3, STD4, STD5, STD6), assign concentrations (1E+06, 1E+05, 1E+04, 1E+03, 1E+02, 1E+01 copies/μL) and select "copies/μL" as the unit.

10.3.7 Run PCR Reaction: Open the heated lid, place the eight-strip tubes according to the sample plate setup, close the lid, and start the PCR program.

10.4 Data Analysis

10.4.1 Baseline and Threshold Setting: The instrument will automatically set the baseline and threshold after the reaction end. Save and export the results.

10.4.2 Result Calculation: Import the instrument's detection results into a pre-designed Excel analysis sheet to calculate final detection results. Attach the Excel file to the test records.

10.4.3 Calculation Formula:

Residual K562 Feeder Cell Proportion

= K562 Detection Result ÷ABL Detection Result ×100%

10.5 System Suitability

10.5.1 CV% ⩽ 5% among three replicate wells (Ct values > 35 excluded).

10.5.2 NTC should have no Ct value or a Ct value two cycles higher than the lowest standard curve concentration.

10.5.3 Amplification efficiency: 85.0%~110.0%, with $R^2 \geqslant 0.990$.

10.6 Symbols and Abbreviations

10.6.1 Standard Products: STD;

10.6.2 No Template Control: NTC;

10.6.3 Sample: S

10.7 Matters need Attention

10.7.1 This kit has been verified for stability (freeze-thaw and other factors) without repackaging;

10.7.2 The preparation environment of negative samples and positive samples (reference products and samples to be tested, etc.) should be distinguished from each other, and should not be operated in one area;

10.7.3 Pay attention to the timely replacement of the suction tip between different loading steps to avoid cross-contamination and long-term opening of the cover;
10.7.4 The kit must be used within a valid period;
10.7.5 All components in the kit are recommended to be used after melting in a low temperature environment;
10.7.6 Only by strictly observing the operation method of the instructions, all the reagents matched with this kit can ensure the best detection effect;
10.7.7 The final test results are closely related to the effectiveness of the reagent, the operation method of the operator and the test environment;
10.7.8 This kit is intended for in research use only and is not intended for clinical diagnosis.

## 11. Disclaimer

This product is for research use only and is not intended for use in humans, diagnostics, or therapeutic applications.
Under all circumstances, the company's liability for this product is limited solely to its value.

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