Instructions for Use of PG13 Residual DNA Detection Kit (qPCR)

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

Cat. No. HG-PG001

Introduction

The PG13 cell line was obtained by integrating a Moloney leukemia virus (MLV) expression vector and gibbon leukemia virus (GALV) membrane protein gene modification from mouse embryonic fibroblasts (NIH/3T3). PG13 cells, as a type of packaging cell, are currently mainly used in the development of cellular products such as CAR-T/TCR-T cells. Therefore, relevant biological products need to be tested for the content of residual DNA in PG13 cells.

This kit is equipped with PG13 DNA quantitative reference which can quickly and accurately quantitatively detect PG13 residual DNA in the intermediate, semi-finished and finished products of various biological products.

The kit is equipped with the sample preprocessing kit of our company (Cat. No.: HG-CL100) for sample preprocessing.

The detection range is from 3 fg/μL to 3 × 10^5 fg/μL.

Specification

100 Reactions

Kit components

Table 1: Kit components and storage conditions

Components	Specification	Storage temperature
PG13 DNA quantitative standard (30 ng/μL)	50 μL×1vial	-20℃
PG13 Primer & Probe MIX	550 μL×1vial	
2x qPCR Reaction Buffer	1.6 mL × 1vial	
DNA diluent	1.5mL× 3vial	

Product storage conditions and shelf life

See the above table for storage conditions, and the shelf life is 12 months. After opening, unused kits should be stored Under specified storage conditions.

Applicable model

Including but not limited to ABI7500, BioRad CFX96, Bioer FQD-96A, Roche Light Cycler 480 and other real-time quantitative fluorescence PCR instruments.

Consumables and equipment to be self-prepared

Please prepare the following consumables and equipment before the test

- 1.5 mL or 2 mL sterile low adsorption centrifuge tubes
- 96-well qPCR plate or 8-strip tube adapted to PCR instrument
- 1000 μL, 200 μL, and 10 μL sterile low adsorption pipette tips with cartridge
- Fluorescence quantitative PCR instrument
- Pipettes of various specifications (e.g., 1000 μL, 200 μL, 10 μL, 2.5 μL)
- Centrifuge
- Oscillator
- Magnetic stand
- Water bath/metal bath

Test steps

I. Sample Preprocessing

Please refer to the operating instructions of our sample preprocessing kit (Cat. No.: HG-CL100) for details.

II. qPCR Operation Steps

1. Preparation of quantitative reference and NTC/NCS

1.1 Quantitative reference: Take out DNA quantitative reference and DNA diluent, and thaw on ice; after thawing completely, shake gently to mix well, and centrifuge instantly;

1.2 Take 7 clean 1.5 mL centrifuge tubes and label them as ST0, ST1, ST2, ST3, ST4, ST5, and ST6, respectively;

1.3 The standard dilution process is shown in the table below:

Table 2

Standard No.	Dilution volume	Concentration (fg / μL)
ST0	10μL Quantitative reference + 90 μL DNA diluent	3 x 10^6
ST1	10 μL ST0 + 90 μL DNA diluent	3 x 10^5
ST2	10 μL ST1 + 90 μL DNA diluent	3 x 10^4
ST3	10 μL ST2 + 90 μL DNA diluent	3 x 10^3
ST4	10 μL ST3 + 90 μL DNA diluent	3 x 10^2
ST5	10 μL ST4 + 90 μL DNA diluent	3 x 10^1
ST6	10 μL ST5 + 90 μL DNA diluent	3 x 10^0

1.4 Preparation of NTC: 100 uL DNA diluent;

1.5 Preparation of NCS: Take 100 μL of DNA diluent and sample for sample preprocessing;

1.6 Spike recovery of ERC: It is suggested that 90 uL sample + 10 uL Quantitative Reference 3 can be prepared in other ways according to the actual situation.

2. Preparation and addition of qPCR reaction solution

2.1 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 =(Standard curve with 6 concentration gradients+ 2 negative control(NTC/NCS) +test sample)× 3

2.2 Calculate the total amount of qPCR MIX required for this time based on the number of reaction wells:

qPCR MIX = (Number of reaction wells + 2 or 3) × 20 μL (2 or 3 is operational loss)

2.3 Thaw the reagents to be used on ice, mix by gentle shaking, and prepare the qPCR MIX as shown in Table 3.

Table 3 qPCR MIX Preparation

Components	Volume required for single reaction(μL)
2 × qPCR Reaction MIX	15
Primer&Probe MIX	5
Total volume	20

3. Thaw the required reagents on ice, mix well by gentle shaking, and load as shown in Table 4(total volume of 30 μL):

Table 4. Examples of loading to each reaction well

Standards	20μL qPCR Mix +10μL ST1/2/3/4/5/6
Negative control	20μL qPCR Mix +10μL NTC/NCS
Test sample	20μL qPCR Mix +10μL test sample

4. In the experiment, sterile nuclease-free 8-tube strips or 96-well plates should be used for qPCR experiment, bubbles should be removed from the reaction system, and the liquid should be centrifuged to the bottom of the tube to prepare for the reaction.

5. Layout illustration of reaction wells

Table 5. Layout illustration of Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST1	ST1	ST1							S1	S1	S1
B	ST2	ST2	ST2							S2	S2	S2
C	ST3	ST3	ST3							S3	S3	S3
D	ST4	ST4	ST4									
E	ST5	ST5	ST5									
F	ST6	ST6	ST6							ERC-S1	ERC-S1	ERC-S1
G					NTC	NTC	NTC			ERC-S2	ERC-S2	ERC-S2
H					NCS	NCS	NCS			ERC-S3	ERC-S3	ERC-S3

III. qPCR reaction program and parameter setting

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

1. Create an experimental reaction program and set up a two-step reaction program as shown in the table below.

Table 6. PCR reaction program

Stage1	Pre-denaturation	Reps:1	95℃	30s
Stage2	Cyclic reaction	Reps:40	95℃	5s
			56℃	34s

Note: The reaction volume is 20 μL. Set the program at 56°C for 34s for fluorescence collection; For other models of equipment, if you encounter any problems, you can consult our company or the instrument manufacturer.

2. Create an experimental reaction plate, 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 "PG13-DNA"; then input the number of replicate wells and Sample Name for each sample.

3. Select standard curve wells in the reaction plate chart, select "Standard" in the "Sample Type" dropdown menu, check fluorescent FAM, and name the Target Name as "PG13-DNA "; then input the number of replicate wells and Sample Name for each dilution gradient. Assign values of 3E+05, 3E+04, 3E+03, 3E+02, 3E+01, 3E+00 (in fg/μL), respectively, to the "Concentration" column of ST1, ST2, ST3, ST4, ST5, and ST6.

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

IV. qPCR result analysis

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

1. Click Quantitation in Data Analysis Window to read the slope, intercept, amplification efficiency (Effect) and R^2 of the standard curve.

2. In the window Quantitation Data, the SQ Mean column reads the test values of the no-template control (NTC/NCS) and the test sample in copies/μL.

3. Data reliability evaluation:

- The difference in Ct values between 3 replicate wells shall be less than 1.0, except for wells with Ct value greater than 35;
- The CT values of negative controls NTC and NCS should be greater than the CT value of the lowest concentration of the standard curve, or the criteria should be set based on the laboratory's own validation results;
- Linear correlation coefficient R^2 of the standard curve shall be equal or greater than 0.98, amplification efficiency shall be within 85%-110%;
- The recovery of ERC shall be within 50%-150%(spike recovery = ERC/(0.9*sample + 0.1*Quantitative Reference 3).

Precautions

1. The kit has been validated for the stability (freezing-thawing and other factors) and does not require dispensing.

2. The preparation for negative samples and positive samples (reference and samples to be tested, etc.) should be separated into different environments and should not be operated in one area. The preparation personnel should wear neat masks, gloves and cleanroom garment.

3. Tips shall be changed between different loading steps in time to avoid cross-contamination and long-time opening.

4. The kit must be used within the shelf life.

5. All components in the kit are recommended to be used after melting in a low temperature environment.

6. The best detection effect can be ensured only by strictly following the instructions and using all the reagents provided with this kit.

7. Subsequent qPCR detection shall be performed immediately after sample preprocessing and purification as far as possible on the same day to ensure the accuracy of test results.

8. The final test results are closely related to the efficacy of reagents, operator's operation methods and test environment.

9. Our company is only responsible for the kit itself and not for the sample consumption caused by the use of the kit. Users should fully consider the possible usage of sample before use and reserve sufficient samples.

10. This kit is for in vitro research use only and is not used for clinical diagnosis.

Disclaimer

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

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