Instructions for Use of BaEV Gene Copy Number Detection Kit (qPCR)

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

Cat. No. HG-BA001

Introduction

The BaEV Gene Copy Number Detection Kit is a specialized kit for quantitative detection of BaEV gene copy number.

This kit quantitatively detects the copy number of BaEV gene in the sample based on the fluorescence probe method.

This kit is rapid, specific and reliable in performance.

The kit is equipped with the genomic DNA extraction kit of our company (Cat. No.: HG-NA100) for sample preprocessing. Detection range : 2×10^{1} copies/μL to 2×10^{6} copies/μL.

Specification

100 Reactions

Kit components

Table 1: Kit components and storage conditions

Components	Specification	Storage temperature
BaEV quantitative standard (2×10^{8} copies/μL)	50 μL×1vial	-20℃
BeEV Primer&Probe MIX	550 μL×1vial	
2x qPCR Reaction Buffer	1.2 mL × 1vial	
DNA diluent	1.5mL× 3vial	
ROX High	50 μL×1vial	
ROX Low	50 μL×1vial	

Product storage conditions and shelf life

See the above table for storage conditions, and the shelf life is 18 months.

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. (Table2)

Instruments	ROX reference stain
Applied Biosystems® 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™	ROX High
Applied Biosystems® 7500, ViiA™ 7, QuantStudio™ 12K Flex, Agilent Mx3000P™, Mx3005P™, and Mx4000™	ROX Low
Rotor-Gene™, DNA Engine Opticon™, Opticon™ 2, Chromo 4™ Real-Time Detector, Mastercycler® ep realplex, Smart Cycler®, Roche LightCycle®480, Roche LightCycler®Nano, Bio-Rad CFX96, and Illumina Eco™	No ROX

Note:Select an appropriate ROX for the model. If the corresponding model is not found in the above table, please consult our company or the instrument manufacturer

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 genomic DNA extraction kit (Cat. No.: HG-NA100) for details.

II. qPCR Operation Steps

1. Preparation of quantitative reference and NTC/NCS

1.1 Quantitative reference: Take out BaEV 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 3

Standard No.	**Dilution volume**	**Concentration (copies/μL)**
ST0	10μL Quantitative reference + 90 μL DNA diluent	2 x 10^7
ST1	10 μL ST0 + 90 μL DNA diluent	2 x 10^6
ST2	10 μL ST1 + 90 μL DNA diluent	2 x 10^5
ST3	10 μL ST2 + 90 μL DNA diluent	2 x 10^4
ST4	10 μL ST3 + 90 μL DNA diluent	2 x 10^3
ST5	10 μL ST4 + 90 μL DNA diluent	2 x 10^2
ST6	10 μL ST5 + 90 μL DNA diluent	2 x 10^1

1.4 Preparation of NTC: 100 uL DNA diluent;

1.5 Preparation of NCS: pre-treat Sample and 100μL DNA Diluent at the same time.

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) × 15 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 4.

Table 4　qPCR MIX Preparation

Components	Volume required for single reaction(μL)
2 × qPCR Reaction Buffer	10
BaEV Primer&Probe MIX	4.6
ROX*	0.4
Total volume	15

* Please select appropriate ROX for corresponding model. If there is no ROX suitable for the model, please add deionized water (free of nucleic acid and nuclease contamination) of same volume.

3. Thaw the required reagents on ice, mix well by gentle shaking, and load as shown in Table 5 (total volume of 20 μL):

Table 5.　Examples of loading to each reaction well

Standards	15μL qPCR Mix +5μL ST1/2/3/4/5
Negative control (NTC)	15μL qPCR Mix +5μL NTC/NCS
Test sample	15μL qPCR Mix +5μL 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 6.　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 7.　PCR reaction program

Stage1	Pre-denaturation	Reps:1	95℃	2min
Stage2	Cyclic reaction	Reps:40	95℃	15s
			60℃	30s

Note: The reaction volume is 20 μL. Set the program at 60℃ for 30s for fluorescence collection; For other models of equipment, if you encounter any problems, you can consult our company or the instrument manufacturer.

2. Create the experimental reaction plate, click Select Fluorophores to select the fluorescence FAM; in the reaction plate diagram, select the Sample well, pull down in Sample Type to select Unknown, check the fluorescence FAM, Target Name is designated as BaEV; input the number of replicates for each sample and Sample Name.

3. In the reaction plate diagram, select the Standard well, pull down in Sample Type to select Standard, check the fluorescence FAM, and Target Name is designated as BaEV; input the number of replicates for each dilution gradient and Sample Name. And the Concentration column of ST1, ST2, ST3, ST4, ST5 , ST6 is assigned values of 2.00E+06, 2.00E+05, 2.00E+04, 2.00E +03, 2.00E+02, 2.00E+01(in copies/uL), respectively.

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*ST3)

4. Criteria for negative BaEV result: There is no Ct value in 3 replicate wells of the sample or no Ct value in 2 of the 3 replicate wells and there is Ct value in 1 replicate well of the sample, and the Ct value of detected well is greater than the Ct value of LLOQ.
5. Criteria for positive BaEV result: If the copy number of BaEV gene detected in 2 or more wells of the sample is not less than 10 copies/μL, it is determined as positive for BaEV gene.

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.