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.

Specification

100 Reactions

Kit components

Components	Filling volume	Storage conditions
BaEV quantitative reference (2 x 10^8 copies/μL)	50 μL x 1 tube	-20℃ and below
BaEV Primer&Probe MIX	550 μL x 1 tube	-20℃ and below, protected from light
2x qPCR Reaction Buffer	1.2 mL x 1 tube	-20℃ and below
DNA diluent	1.5 mL x 3 tubes	-20℃ and below
ROX High (used for background calibration of some instruments, optional per customer request)	50 μL x 1 tube	-20℃ and below, protected from light
ROX Low (used for background calibration of some instruments, optional per customer request)	50 μL x 1 tube	-20℃ and below, protected from light

* Please select the appropriate ROX for the model.

Storage conditions and shelf life

Unopened kits are stored for 12 months at -20℃ and below.

User-provided materials

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
- Quantitative fluorescence PCR instrument
- Centrifuge
- Oscillator
- Pipettes of various specifications (e.g., 1000 μL, 200 μL, 10 μL, 2.5 μL)

Adaptive models (including but not limited to)

- ABI7500
- BioRad CFX96
- Bioer FQD-96A
- Roche Light Cycler 480

When used with different models, please pay attention to select the suitable ROX reference dyes.

Instruments	ROX reference dyes
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

Operating steps

I. Conduct 10-fold gradient dilution of BaEV quantitative reference, and the specific operation is as follows:

1. Take out BaEV quantitative reference and DNA diluent and place them in a 4℃ refrigerator or on ice for melting; after completely thawing, slightly shake and mix well, and perform instantaneous centrifugation.

2. Take 7 clean 1.5 ml centrifuge tubes and respectively label them as ST0, ST1, ST2, ST3, ST4, ST5 and STD6.

Reference standard code	Dilution volume	Concentration (copies/μL)
STD0	10 μL reference + 90 μL DNA diluent	2.00 x 107
STD1	10 μL STD0 + 90 μL DNA Diluent	2.00 x 106
STD2	10 μL STD1 + 90 μL DNA Diluent	2.00 x 105
STD3	10 μL STD2 + 90 μL DNA Diluent	2.00 x 104
STD4	10 μL STD3 + 90 μL DNA Diluent	2.00 x 103
STD5	10 μL STD4 + 90 μL DNA diluent	2.00 x 102

STD6	10 μL STD5 + 90 μL DNA Diluent	2.00 x 101

II. Preparation and sample addition of qPCR reaction solution

1. According to the standard curve to be detected and the number of samples to be tested, calculate the number of reaction wells required, and generally make 3 replicate wells per sample.

Number of reaction wells = (standard curve for 6-concentration gradient + 1 no template control + number of samples to be tested) x 3

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) x 15 μL (including loss of 2 wells)

3. Take out the reagents and melt them on ice, shake slightly and mix well, and add samples as shown in the following table to prepare qPCR MIX:

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

* Please select the appropriate ROX for the corresponding model; if the appropriate ROX for the model is no ROX, please add an equal volume of enzyme-free water.

Add samples to each reaction well as shown in the table below:

Standard curve	15μL qPCR MIX + 5μL STD1/2/3/4/5/6
No Template Control (NTC)	15 μL qPCR MIX + 5 μL DNA diluent
Samples to be tested	15 μL qPCR MIX + 5 μL samples to be tested

After sample addition, the total volume of each well will be 20 μL. Slightly shake the 96-well plate or 8-strip tube and mix well, quickly centrifuge for 10 s and place them into the qPCR instrument.

III. Parameter setting of qPCR program

The following is an example of the BIO-RAD CFX96 qPCR instrument.

1. Create an experimental reaction program and set up a two-step reaction program with the following PCR reaction program:

Pre-denaturation at 95℃ for 2 min;

40 cycles of 95℃ 15s and 60℃ 30s;

Set the reaction volume as 20 μL.

2. Create the reaction plate and click Select Fluorophores to select fluorescence FAM;

a) Select the sample well in the reaction chart, drop down and select Unknow in Sample Type, check FAM, and name Target Name as BaEV; enter the number of replicates per sample and Sample Name.

b) Select the standard curve well in the reaction chart, drop down and select Standard in Sample Type, check FAM, and name Target Name as BaEV; enter the number of replicates for each dilution gradient and Sample Name. Assign values of 2 x 10^6, 2 x 10^5, 2 x 10^4, 2 x 10^3, 2 x 10^2 and 2 x 10^1 (in copies/μL) in the Concentration column of STD1/2/3/4/5/6, respectively.

3. Click Start Run to select the save path.

IV. Analysis of qPCR results

The following is an example of the BIO-RAD CFX96 qPCR instrument.

1. Click the data analysis window Quantitation to read the Slope, Intercept, Effect (amplification efficiency) and R^2 of the standard curve.

2. In the window Quantitation Data, read the BaEV copy number detection value (copies/μL) for the NTC and sample to be tested in the SQ Mean column.

3. NTC test result shall be N/A or Ct greater than Ct mean of the lowest concentration of the standard curve.

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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