USER GUIDE

# ECALBIO® Bovine Viral Diarrhoea qPCR Kit

**CAT#: ARTS1002**

**ECALBIO CO., LTD**

Email: info@ecalbio.com

Tel: 86-27-87745856

HZAU, Luoshi Road, Wuhan City 430075, China

## 1 Principle And Intended Use

This kit is based on Fluorescence Quantitative Polymerase Chain Reaction (PCR) , and the reaction is in the same PCR tube and it can effectively avoid the contamination. It can rapidly detect the gene of Bovine Viral Diarrhoea in the animal original food, meat, feed, blood, and other samples with high specificity, sensitivity and easy operation.

## 2 Specifications

100 tests/kit

## 3 Components

| Component | Amount | Storage |
|---|---|---|
| Bovine Viral Diarrhoea qPCR Master MIX | 1150 μL × 2 | -20 ℃ |
| Bovine Viral Diarrhoea Positive Control | 100 μL | -20 ℃ |
| Negative Control | 200 μL | -20 ℃ |

## 4 Storage Condition and Expiration Date

① The reagents are stable when stored at -20 ℃ for long time without packing.
② The kit is transported at 2 ℃ to 8 ℃.
③ The kit should remain sealed and away from light exposure.
④ Do not use the kit beyond the expiration date.

## 5 Applicable Instruments

- ❖ Bio-Rad CFX96 Touch Real-Time PCR Detection System;
- ❖ Applied Biosystems™ 7500 Real-Time PCR system;
- ❖ Applied Biosystems™ Quant Studio 5 Real-Time PCR system;
- ❖ Applied Biosystems™ QuantStudio 3 Real-Time PCR system;
- ❖ Applied Biosystems™ QuantStudio 7 Flex/Pro Real-Time PCR system;
- ❖ Applied Biosystems™ ViiA7 Real-Time PCR system.

## 6 Materials Required but Not Provided

① Real-time PCR instrument
② Adjustable micropipettors (10 µL, 100 µL)
③ Benchtop microcentrifuge with adaptors for PCR
④ Laboratory mixer (Vortex mixer or equivalent)
⑤ Optical reaction plates and covers, or optical PCR tubes and caps
⑥ Aerosol-resistant pipette tips
⑦ 1.5-mL nuclease-free microcentrifuge tubes
⑧ Powder-free disposable gloves
⑨ Nuclease-free water
⑩ ECALBIO® Bacteria DNA Purification Kit (CAT#: D2512)

## 7 Samples collection and DNA extraction

① Samples collections

Sample collection is according to some international standards or national standards.

② DNA extractions

Use ECALBIO® Bacteria DNA Purification Kit (CAT#: D2512) to extract high quality DNA of Salmonella .

③ Prepare analysis

Prepare at least one mock sample as a negative extraction control, processed with the same DNA isolation method that is used for test samples.

Dilute the final DNA sample to 10 ng/µL for the PCR.

## 8 Set up the PCR reactions

① Mix thoroughly by vortex, then distribute 23 μL to each reaction well or tube.

② Add 2 μL of DNA sample, mock-purified sample (negative extraction control), Negative Control (or no-template control), Positive Control to the appropriate wells.

③ Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom.

## 9 PCR Assay

① Create a new experiment.

② See the appropriate instrument user guide for detailed instructions to set up and run the real-time PCR instrument.

③ Select "none" for Passive Reference (For ABI Real-Time PCR systems).

④ Set cycle conditions. Enter the reaction volume (25$\mu L$), and edit the PCR program as follows:

Step 1: 95℃, 30 s;

Step 2: 95℃, 5 s;

Step 3: **58℃**, 30 s (fluorescence collection ). (40 cycles)

Save the file. Run the program.

⑤ Load the reactions, run the thermal cycle program and collect real-time amplification data.

## 10 Analysis of Results

① The general process for analyzing results is described in this section. The details of data analysis depend on the real-time PCR instrument that you use; refer to the appropriate user guide for instructions on how to analyze your data.

② View the amplification plots for all reactions to make sure that they appear normal.

③ Use the Auto instrument setting to set the baseline.

④ Check that the results obtained in all control wells are as expected.

| Reaction type | SYBR channel (Salmonella DNA) | Ct Value |
|---|---|---|
| Positive control | + | ≤30 |
| Negative extraction control | - | > 35 or NA |
| Negative control | - | > 35 or NA |

## 11 Assign and interpretation results

| Sample Ct Value | TM value | Sample result* | Interpretation |
|---|---|---|---|
| Ct ≤ 30 | 79.0 [a] | Positive ( + ) | Bovine Viral Diarrhoea DNA detected. |
| Ct > 35 | NA [b] | Negative ( - ) | Bovine Viral Diarrhoea DNA not detected. |
| 30 < Ct ≤35 [c] | -- | Suspicious ( + - ) | Bovine Viral Diarrhoea DNA may be detected. |

**a.** Tm value is consistent with the positive control Tm value is inconsistent with the Positive Control.

**b.** If 30 < Ct value ≤35, sample is suspect. For the suspicious samples, check amplification curve and Tm value. If the amplification curve is logarithmic.

**c.** Amplification curve, and the Tm value is consistent with the positive control, it was suspicious positive, otherwise judged as negative.

## 11 Good laboratory practices for PCR

To avoid amplification contamination of samples, follow these guidelines when preparing or handling samples for PCR amplification as following.

① Maintain separate areas and dedicated equipment and supplies for : Sample preparation reaction setup amplification and analysis of products.

② Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation). Change gloves whenever you suspect that they are contaminated

③ Do not bring amplified products into the reaction setup area.

④ Open and close all sample tubes carefully. Avoid splashing or spraying samples. Keep reactions and components capped as much as possible.

⑤ Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.

⑥ Do not open reaction tubes after PCR.

⑦ Do not autoclave reaction tubes after PCR.

⑧ Clean lab benches and equipment periodically with 10% bleach solution or other solutions. After cleaning with bleach we recommend a rinse with distilled water or an ethanol solution because bleach will rust stainless steel.

⑨ Note that minor discoloration of metal parts may occur.

## 12 Limitations of the Procedure

① The test results of this kit are only for research reference. The clinical diagnosis and treatment of patients should be considered in combination with clinical observations, patient history, and epidemiological information.

② The test results may be affected by the quality of the samples collected as well as their handling, transportation and storage. Deficiencies in these factors may lead to false negative results.

③ False positive results may occur if cross-contamination is not controlled during sample processing.

④ Amplification of Endogenous Control may fail if pathogen concentration in the specimen is high.

⑤ Nucleic acid extraction reagents from other companies need to be tested on several samples to verify their suitability.

## 13 Precaution

① For research use only, not used in human therapy.

② Read instructions carefully before testing. The test must be performed according to the instructions provided.

③ Laboratory management shall be strictly by management standards of the nucleic acid test laboratory.

④ Conduct quality control for every experiment.

⑤ Pipettes used with this test must be calibrated regularly.

⑥ The entire process should be divided into three separate areas within the laboratory: the first area for reagent preparation, the second area for specimen processing and reaction system preparation and the third area for amplification.

⑦ Fluorescence detection and result analysis. The instruments, equipment, and personal protective equipment (PPE) in each area should be used independently to prevent cross-contamination.

⑧ Operators of the test should always avoid potential contamination of RNase and DNase. Test operators should not directly touch the reaction tube by hand. Operators must use disposable gloves and testing materials without Fluorescent properties.

⑨ Follow biohazard safety standard precautions. Biological safety cabinets should be used when handling specimens to ensure the safety of operators and prevent pollution. Harmful and/or toxic specimens and reagents should be properly stored and maintained by designated personnel. Waste should be properly disposed of in special containers. Instruments and equipment such as operating tables, pipettes, centrifuges, and amplification instruments should be frequently wiped and disinfected with 10% sodium hypochlorite and/or 75% ethanol. Laboratory rooms and ultra-clean workbenches should be regularly treated with UV lamps after each experiment.

⑩ The reagents in the centrifuge tube should be fully thawed and mixed before use. Centrifuge for a few seconds to concentrate the liquid at the bottom of the centrifuge tube. When preparing the reaction system, it should be noted that the mixing of all liquids should be carried out on the vortex mixer as much as possible. Once the reaction system is completed, centrifuge for a few seconds at a low speed.

⑪ Do not mix reagents from different batches.

*Ecalbio makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, ecalbio will provide a replacement product. There is no warranty of merchant ability of this product , or of the fitness of the product for any purpose. ecalbio shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.*

**ECALBIO CO., LTD**
**Web:** www.ecalbio.com
**Email:** info@ecalbio.com
**Tel:** 86-27-87745856
**Add:** HZAU, Luoshi Road, Wuhan City 430075, China