Bovine viral diarrhea virus (BVDV) Detection Kit

Product Number: DTL1148

Shipping and Storage

1. Store below 30°C. It is valid for 12 months.
2. Transport at normal temperature, not suggested over 14 days.
3. Opened but not completely used the all components should be stored at (-20±5)°C. It is recommended to separate in PCR tubes before refrigeration to avoid repeated freezing and thawing of all reagents next time. It is not recommended to repeat the freeze-thaw cycle more than 7 times.
4. Date of manufacture and term of validity: see the label.

Component

Component	48T
BVDV RT-PCR Master Mix	Lyophilized powder ×1 Bottle
Exogenous Internal Control	Lyophilized powder ×1 Bottle
Positive Control	100μL
Negative Control	1mL
Redissolved Diluent	1.5mL

1. Do not mix reagents from different batches.
2. The reaction system is lyophilized powder that contains all components required for fluorescence PCR, including Taq enzyme, reverse transcriptase, primers, probes, dNTPs, and Mg^{2+}.

Description

Bovine viral diarrhea is an infectious disease caused by bovine viral diarrhea virus (BVDV), which can cause many clinical symptoms and some diseases, including reproductive disorders, respiratory syndrome, congenital defects, enteritis, persistent infection (PI) and mucosal disease (MD). In addition to cattle, susceptible animals also include camels, sheep, goats, pigs, deer and a variety of wild ruminants. The secretions, excrement, blood and spleen of sick cattle contain viruses, which are transmitted by direct or indirect contact.

This kit is suitable for detecting bovine viral diarrhea virus nucleic acid in bovine tissue samples, nasal swabs and other specimens, and is suitable for auxiliary diagnosis of bovine viral diarrhea virus infection.

Application

This kit uses TaqMan probe real-time fluorescence PCR technology to design a pair of bovine viral diarrhea virus-specific primers, combined with a specific probe, and uses fluorescence PCR technology to perform in vitro amplification and detection of bovine viral diarrhea virus nucleic acid, which is used for clinical etiological diagnosis of suspected infected samples.

Applicable instruments

Real-time fluorescence PCR instrument with VIC,FAM channels.

Specimen collection

1. Applicable sample type: Tissues sample or Nasal swab sample.
2. Sample collection: Tissue samples: Use sterile scissors and forceps to collect organs or tissues to be examined. Intestinal mucosal scrapings or lungs, spleens, lymph nodes, etc. can be collected as tissue samples to be examined. For aborted fetuses and stillbirths, fetal tissues can be collected directly. For cattle suspected of dying from mucosal diseases, blood clots or tissues such as lungs, spleens, lymph nodes, etc. can be collected, especially intestinal collected lymphoid tissue. If the intestinal

sample has been autolyzed, tonsils and lymph nodes can be collected. Put the collected samples into sterile sampling bags or other sterilized containers; Nasal swab sample: For cattle suspected of acute infection or persistent infection, collect nasal secretion swabs. When taking the nasal swab, swab deep into the nasal cavity, wipe back and forth 2-3 times and rotate, put into a sampling tube containing 2mL PBS. Then dilute 4 times with cell culture medium containing 1000U/mL penicillin and streptomycin, centrifuge at 2000r/min for 15min, take the supernatant, and number it for use.

3. Then using sterile scissors and tweezers to cut 2.0g sample to be examined in a mortar or tissue homogenate fully grinding, and then add 10mL PBS mixing, 2000r/min after centrifugation for 5 min, collect 1mL supernatant into a sterile 1.5mL sterile centrifuge tube, numbered for use.
4. Sample storage and transportation: The collection or processing sample should not exceed 24 hours under the conditions of 2℃ ~ 8℃. If long-term preservation is needed, it should be stored below -70℃, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

1.1. Take out the BVDV RT-PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 960μL of Redissolved Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30 ~ 60 seconds until the liquid is clear and transparent. Subpackage it into PCR reaction tubes according to 19μL/ tube.

1.2. Take out the Exogenous Internal Control and open the tube cap,add 100μL of Redissolved Diluent,mix evenly for 15 seconds with a vortex shaker, centrifuge for 3 seconds with a palm centrifuge, and then subpackage it into PCR reaction tubes according to 1μL/ tube.

The total test reaction system mixed well between the BVDV RT-PCR Master Mix and Exogenous Internal Control is 20μL/tube.

2. Nucleic acid extraction:

This kit is not included for Nucleic Acid(NA) extraction reagent.

Commercially available extraction kits that have been shown to generate highly purified RNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted RNA is not used immediately, it should be stored below -20℃. For long-term storage, it should be stored below -80℃ and avoid repeated freezing and thawing.

Note: The Negative Control and the Positive Control does not require nucleic acid extraction.

3. Add sample:

The correspond substances were added to that above PCR reaction tubes according to the following table:

Type	Add sample description
Testing Sample	Add 5μL of the extract prepared in step 2 to the reaction tube, and close the tube cover.
Negative Control/ Positive Control	Add 5μL of Negative Control and Positive Control to the reaction tube separately, and cover the tube tightly.

The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be mixed well and centrifuged for 5s on a palm centrifuge and then delivery to the nucleic acid amplification region. If bubbles are found, the tube wall should be gently flicked to remove bubbles and centrifuged again.

4. PCR amplification:

Place the reaction tube in the automatic fluorescent PCR instrument, set the negative control, positive control, and test sample parameters to perform PCR experiment according to the operating instructions of the instrument, and record the corresponding sample name.

Select FAM channel to detect BVDV RNA,select VIC channel to detect Internal control. Set the sample reaction system to 25μL.

(Note: For ABI series instruments, select ‘None’ under ‘Quencher’, and select ‘None’ as the dye to use as the passive reference.)

Recommended reaction program setting:

Step	Cycles	Temperature	Time	Collect fluorescence signal
1	1 cycle	50℃	10min	No
2	1 cycle	95℃	2min	No
3	45 cycles	95℃	15sec	No
		60℃	30sec	Yes

5. **Result analysis:**

After the reaction is completed, the results are automatically saved.

The Start value, End value and Threshold value of the Baseline should be adjusted according to the analyzed image (the user can adjust it according to the actual situation, the Start value can be set at 3-15, the End value can be set at 5-20, the amplification curve of the negative control should be adjusted to be flat or below the threshold line).

Click Analyze for analysis, make the parameters meet the requirements in the following '6.Quality control', and then go to the Plate window to record the Ct value.

6. **Quality control**

Negative Control: FAM detection channel has no obvious amplification curve.

Positive Control: FAM detection channel has an obvious amplification curve, and the Ct value ≤32.00. Exogenous Internal Control: VIC detection channel has an obvious amplification curve, and the Ct value ≤32.00.

The above requirements must be met at the same time in the same experiment, otherwise this experiment is invalid and needs to be repeated.

Explanation of Test Result

1. Positive:Ct value ≤40.00 and the curve has a clear index growth curve.
2. Negative:The sample test results have no Ct value and no specific amplification curve, IC Ct value ≤40.00.
3. Suspicious samples: If the sample test result is 40.00<Ct value≤45.00, it is recommended to repeat the test. If the test channel is still 40.00<Ct value≤45.00 and the curve has an obvious growth curve, it is judged as positive, otherwise it is negative.

Limitation

1. Sample detection results are related to sample collection, processing, transportation and preservation quality.
2. If cross-contamination is not controlled during the sample extraction process, false positive results will occur.
3. Positive control and leakage of amplification products can lead to false positive results.
4. The genetic mutations and reorganizations during epidemics can lead to false negative results.
5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Reagent transportation, improper preservation, or inaccurate reagent preparation reagent detection performance decreases, and the results of false negative or quantitative detection occur.
7. The results of this test are for reference only. If the diagnosis must be confirmed, please combine clinical symptoms and other test methods.

Performance Parameters

1. Limit of Detection: The minimum detection limit of this reagent is 500 copies/mL.
2. Precision: Repeat detection of the enterprise precision reference product 10 times, and the coefficient of variation (CV, %) value of detected concentration logarithm is ≤5.00%.
3. Compliance rate of negative/positive reference products: The compliance rate of negative reference products in enterprise reference is 100%, and the compliance rate of positive reference products is 100%.

Note

1. Please read the instructions of this kit carefully before the experiment, and strictly follow the operation steps.
2. Before the test, please be familiar with and master the operation method and precautions of various instruments to be used, and

carry out quality control for each experiment.

3. The reaction solution should be stored away from light.
4. Try to avoid bubbles in the reaction, and the tube cover needs to be tight.
5. Use disposable heads, disposable gloves and special work clothes in each district.
6. Sample processing, reagent preparation, and samples need to be performed in different areas to avoid cross -pollution.
7. After the experiment is completed, use 10% hypochloride or 75% alcohol or ultraviolet light to treat the workbench and pipette.
8. All items in the kit should be treated as pollutants and processed in accordance with the "Biological Safety General of Microbiological Biomedical Laboratory".