

Viral haemorrhagic septicaemia virus(VHSV) Detection kit

Product Number: DTL1224

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
VHSV RT-PCR Mater Mix	Lyophilized powder ×1 Bottle
Exogenous Internal Control	Lyophilized powder ×1 Tube
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 Mg²⁺.

Description

This kit designs specific primers and probes for viral hemorrhagic septicemia virus genes, and uses one-step fluorescent RT-PCR technology to perform in vitro amplification and detection of viral hemorrhagic septicemia virus RNA for etiological diagnosis of suspected infected samples.

Application

Viral hemorrhagic septicemia is a highly fatal disease caused by viral hemorrhagic septicemia virus (VHSV), which belongs to the genus Rhabdovirus of fishes. VHSV contains 3 serotypes and 4 genotypes, and there is no correlation between serotypes and genotypes. The disease first became prevalent in continental Europe, mainly infecting rainbow trout, flatfish, and several wild marine fish. The disease shows obvious clinical symptoms during the acute infection period: sick fish are lethargic, the skin turns black, the eyes are protruding, anemia, bleeding at the base of fin rays, gills, eyes, and skin, abnormal swimming, and abdominal edema. The main transmission route of the virus is contact with other infected fish or contaminated water, pollutants or through fish-eating birds.

This kit is suitable for detecting viral hemorrhagic septicemia virus RNA in specimens such as the brain, liver, kidney, spleen, gills and other tissues of fish, and ovarian fluid of female fish, and is suitable for auxiliary diagnosis of viral hemorrhagic septicemia virus infection.

Applicable instruments

Real-time fluorescence PCR instrument with FAM channel and VIC channel.

Specimen collection

1. Applicable sample type: For fish with clinical symptoms, if the body length is not more than 4cm, take the whole fish, and for fish with a body length of 4-6cm, collect tissue samples of viscera (including kidneys). For fish with a body length greater than 6cm, take brain, liver, kidney, and spleen; for fish without symptoms, take kidney, spleen, gill and brain tissues, and mature

female fish also need to take ovarian fluid.

2. Sample collection: Tissue sample: Weigh about 1 g of sample from 3 different locations of each tissue, chop it with surgical scissors and mix it, then take 0.5g and grind it in a grinder, add 1.5mL of normal saline and continue grinding. After homogenization, transfer it to a 1.5mL sterile centrifuge tube, centrifuge it at 8000 rpm for 2 min, and take 100µL of the supernatant into a 1.5mL sterile centrifuge tube for use; Ovarian fluid sample: 100µL of ovarian fluid was directly collected for nucleic acid extraction.
3. Sample storage and transportation: The collection or processing sample should not exceed 24 hours under the conditions of 2°C ~ 8°C. If long-term preservation is needed, it should be stored below -70°C, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

- 1.1. Take out the VHSV RT-PCR Mater 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, and then 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, then cover the tube, 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 VHSV RT-PCR Mater 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°C. For long-term storage, it should be stored below -80°C 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, 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 RV nucleic acid, select VIC Channel to detect Internal Control. Set the Reaction Volume per Well 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°C	10min	No
2	1 cycle	95°C	2min	No



3	45 cycles	95°C	15sec	No
		60°C	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 FAM channel of sample test results have no Ct value and no specific amplification curve, and the VIC channel is ≤ 32.00 .
3. Suspicious samples: If the sample test result is $40.00 < \text{Ct value} \leq 45.00$, it is recommended to repeat the test. If the test channel is still $40.00 < \text{Ct value} \leq 45.00$ 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 for VHSV 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 $\leq 5.00\%$.
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.



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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".