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# Torque Teno Sus Virus Type 1/2 (TTSuV1/2) Multiple Detection kit

**Product Number: DTL1511**

## Shipping and Storage

1. Store below 30℃. 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)℃. 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 |
|---|---|
| TTSuV1/TTSuV2 PCR Master Mix | Lyophilized powder ×1 Bottle |
| Exogenous Internal Control | Lyophilized powder ×1 Tube |
| Positive Control | 100μL |
| Negative Control | 1mL |
| Redissolved Diluent | 1.5mL |

Note: Do not mix reagents from different batches.

.The reaction system is lyophilized powder that contains all components required for fluorescence PCR, including Taq enzyme, reverse transcriptase, primers, probes, dNTPs, and $Mg^{2+}$ etc.

## Description

This kit uses specific primers and matched fluorescent probes for Torque Teno Sus virus (TTSuVs). Real-time fluorescent PCR is applied for the in-vitro amplification and detection of TTSuV DNA, for the etiological diagnosis of clinical specimens with suspected infection.

## Application

Torque Teno Sus viruses (TTSuVs) are small, non-enveloped, single-stranded closed circular DNA viruses with high genetic diversity. Based on nucleotide sequence differences, TTSuVs are divided into two genotypes: TTSuV1 and TTSuV2. TTSuV1 belongs to the genus Iotatorquevirus, which contains two distinct genotypes, TTSuV1a and TTSuV1b. TTSuV2 belongs to the genus Kappatorquevirus, comprising TTSuVk2a and TTSuVk2b.

TTSuVs can infect both healthy and diseased pig herds. Co-infection of TTSuVs with Porcine Circovirus Type 2 (PCV2) may trigger Post-Weaning Multisystemic Wasting Syndrome (PMWS). Mixed infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) can lead to Porcine Dermatitis and Nephropathy Syndrome (PDNS). However, its pathogenic mechanism remains unclear.

This kit is suitable for the detection of TTSuV DNA in specimens such as lymph nodes, spleen, lung and serum, and is intended for the auxiliary diagnosis of TTSuV infection.

## Applicable instruments

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

## Specimen collection

1. Applicable sample type: porcine serum, lymph node, spleen and lung tissues.
2. Sample collection: Serum sample: Collect 5mL venous blood from the tested pig using a sterile syringe and transfer it into a

sterile centrifuge tube. Centrifuge at 2000-3000 r/min for 10 min. Transfer the supernatant into a new centrifuge tube for later use. No further pre-treatment is required; the sample can be used directly for nucleic acid extraction. Tissue sample: For dead or euthanized pigs, collect tissue samples such as lymph nodes, spleen and lung. Weigh 1g of tissue, mince it thoroughly, add 2mL of normal saline and grind to prepare tissue homogenate. Centrifuge at 8000 r/min for 5 min, and collect the supernatant for subsequent nucleic acid extraction.

3. 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°C, and the freezing fusion should not exceed 3 times.

## Protocol

**1. Reagent preparation:**

1.1. Take out the TTSuV1/TTSuV2 PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 1mL 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 TTSuV1/TTSuV2 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 DNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted DNA 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, and cover the tube tightly. |

The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be 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 TTSuV1 DNA,select VIC channel to detect TTSuV2 DNA, select ROX 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 | 95℃ | 2min | No |
| 2 | 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 and VIC detection channel has no obvious amplification curve.

Positive control: FAM and VIC detection channel has an obvious amplification curve, and the Ct value ≤32.00. Exogenous Internal Control: ROX 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. The FAM channel is the detection result of Torque teno sus virus type 1,the VIC channel is the detection result of Torque teno sus virus type 2,and the ROX channel is the detection result of Internal Control.
2. Positive:Ct value ≤40.00 and the curve has a clear index growth curve.
3. Negative:The FAM,VIC channel of sample test results have no Ct value and no specific amplification curve,and the ROX channel is ≤32.00.
4. 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. Contamination from the Positive Control or amplified products (amplicons) can lead to false positive results.
4. Genetic mutations or rearrangements in the target sequence may lead to false negative results.
5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Degradation of reagent performance due to improper transportation, storage, or inaccurate preparation may lead to false negative results or inaccurate quantification.
7. The results of this test are for reference only. Clinical diagnosis should be based on a combination of clinical symptoms, other laboratory findings, and epidemiological data.

## Performance Parameters

1. Minimum detection limit: The minimum detection limit of this reagent is 500 copies/mL.
2. Precision: The coefficient of variation (CV, %) of the Ct value of the pathogen detection channel 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% hypochlorite 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".