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Diagnostic Kit for Zika Virus and Dengue Virus and Chikungunya Virus RNA

Product Number: DTL0316

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
ZIKV/DGV/CHIKV RT-PCR Master Mix	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

This kit uses real-time fluorescence PCR to detect Zika virus (ZIKV), Dengue virus (DGV) and Chikungunya virus (CHIKV) with one pair of primers and one specific fluorescent probe respectively. The method was used for the qualitative detection of Zika virus, Dengue virus and Chikungunya virus in serum samples. The kit is provided with an internal control, which can monitor whether there is PCR inhibitor in the sample to be tested by detecting whether the internal control is normal or not, so as to avoid false negative PCR.

Application

This kit is intended for the in vitro qualitative detection of Zika virus, Dengue virus and Chikungunya virus nucleic acids in human serum samples, and it is suitable for the auxiliary diagnosis of diseases caused by Zika virus, Dengue virus and Chikungunya virus.

Zika virus disease, also known as Zika fever, is a viral disease caused by the Zika Virus (ZIKV) and transmitted by mosquitoes. Symptoms: mild fever, rash, conjunctivitis, muscle and joint pain, general malaise, and headache, etc.

Dengue fever (DF) is an acute febrile disease caused by Dengue virus (DGV) and it is characterized by fever,arthralgia, muscle pain, rash, lymphadenopathy, leukopenia, and bleeding, shock and even death in severe cases. The genome of dengue virus is single-stranded positive-stranded RNA, and there are four serotypes (DGV-1, DGV-2, DGV-3 and DGV-4). All four serotypes can cause infection and epidemic of dengue fever, and there is crossover in antigenicity between each type of virus.

Chikungunya fever is an acute infectious disease caused by Chikungunya virus (CHIKV) and transmitted by aedes mosquitoes. The main onset symptoms include sudden onset of fever, chills, trunk rash, severe arthralgia and headache, etc., which may be accompanied by nausea, vomiting, photophobia, conjunctival congestion, abdominal pain or bleeding. Common laboratory tests for Zika virus, dengue virus and chikungunya virus infections include routine blood tests, blood biochemical tests, pathogenic and serologic tests.

Applicable instruments

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

Specimen collection

1. Applicable sample type: serum sample.
2. Sample collection: Use a sterile syringe to draw 2mL of the subject's venous blood and inject it into a sterile centrifuge tube. Leave it at room temperature for no more than 4 hours. Centrifuge at 2000rpm for 5 minutes. Take the upper serum (be careful not to bring in red blood cells) and transfer it to another sterile centrifuge tube for use.
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:

Take out the ZIKV/DGV/CHIKV RT-PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 960μL of Redissolved Diluent, stand for 30 to 60 seconds until the liquid is clear and transparent. 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 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. The Positive Control needs to be redissolved with 100μL of redissolved diluent and mixed well before use.

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. RT-PCR amplification:

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

Select FAM channel to detect DGV nucleic acid, select VIC channel to detect CHIKV nucleic acid,select ROX channel to detect ZIKA nucleic acid,select CY5 channel to detect the 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℃	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, VIC, ROX, CY5 channels has no obvious amplification curve.Internal control will not get amplified in the Negative Controls.Ignore the CY5 amplification graph if a late signal appears in the Negative control wells.

Positive Control: FAM, VIC, ROX, CY5 channels all has an obvious amplification curve, and the Ct value ≤35.00. 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 Dengue Virus detection result, the VIC channel is the Chikungunya Virus detection result, the ROX channel is the Zika Virus detection result, and the CY5 channel is the Internal Control detection result.
2. Positive: The Ct value of the detection channel is ≤40.00, and the curve has an obvious exponential growth curve.
3. Negative: The Ct value of the detection channel is >40.00 or no Ct value, and IC Ct value ≤40.00.
4. If the amplification curve of the FAM channel, VIC channel, and ROX channel of the test sample does not have a logarithmic growth period, and the Ct value of the CY5 channel is >35.00, the test result of the sample is invalid, and the cause should be found and eliminated. Collect samples again and repeat the experiment.

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 ZIKV/DGV/CHIKV virus is 500 copies/mL.
2. Precision: Repeat detection of the enterprise precision reference product 10 times, and 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%.
4. Analysis of specificity:

4.1. Cross-reaction: There is no cross-reaction with human cytomegalovirus, Hepatitis A virus, Hepatitis B virus, Adenovirus, Herpes simplex virus type 1, Herpes simplex virus type 2, Varicella-zoster virus, Neisseria gonorrhoeae, Human herpes

virus type 6, Staphylococcus aureus, Candida albicans.

4.2. Interfering substances: The therapeutic drugs present in the sample,triamcinolone acetonide ≤1mg/L, epinephrine ≤1mg/L, levofloxacin, ceftriaxone ≤25mg/L, did not interfere with the test results of the kit. When the mucin concentration in the sample does not exceed 0.1%, and the volume ratio of blood in the sample does not exceed 20%, there is no interference with the test results of the kit.

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".