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Diagnostic Kit for Influenza A/Influenza B RNA

Product Number: DTL0711

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 BA PCR Master Mix 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. Storage time should not exceed 21 days.
4. Date of manufacture and term of validity: see the label.

Component

Component	48T
Flu A/Flu B 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 the multiplex fluorescent PCR detection technology to to design specific primers and Taqman fluorescent probes for the conserved regions of Influenza A virus and Influenza B virus, so as to realize the qualitative detection of Influenza A virus and Influenza B virus RNA in oropharyngeal swabs. The kit is provided with an Internal Control（IC）, 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

Influenza viruses are divided into four types: A, B, C, and D. Influenza viruses can cause infection and disease in a variety of animals such as humans, poultry, pigs, horses, and bats, and are the causative agent of human and animal diseases such as human influenza, avian influenza, swine influenza, and equine influenza. Typical clinical symptoms of these diseases are acute high fever, generalized pain, significant fatigue and respiratory symptoms. Influenza viruses are mainly spread through droplets in the air, contact between susceptible and infected people, or contact with contaminated items. Generally, autumn and winter are the periods of high incidence.

Applicable instruments

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

Specimen collection

1. Applicable sample type: Oropharyngeal swab.
2. Sample collection: It is advisable to collect samples in the early morning. Rinse the mouth with clean water, assisted by the examiner with a tongue depressor, pass the cotton swab over the root of the tongue to the lesion of the pharyngeal isthmus, apply it several times, avoid touching the tongue and oral mucosa when taking it out. Put the sampled cotton swab into 1mL of normal saline to wash thoroughly, squeeze dry against the container, and discard the cotton swab.

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 Flu A/Flu B RT-PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 960μL of Redissolved Diluent to RT-PCR Master Mix, 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.

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 of Flu A/Flu B RT-PCR Master Mix, 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 Influenza A virus nucleic acid, select ROX channel to detect Influenza B virus nucleic acid, 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,VIC,ROX detection channels has no obvious amplification curve.

Positive Control: FAM,VIC,ROX detection channel 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. Positive: If the Ct value of the detection channel is ≤40.00, and the curve has an obvious exponential growth curve, then the virus type corresponding to the channel is positive, and there is no IC requirement.
2. Negative: If the Ct value of the sample test result is >40.00 or there is no Ct value, and the Ct value of the IC is ≤40.00, then the virus type corresponding to the channel is negative.
3. If the amplification curve of the FAM channel and ROX channel of the test sample does not have a logarithmic growth phase, and the Ct value of the VIC 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. There is no control of cross -pollution during the sample extraction process, and 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, %) 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% 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".