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**【Product Name】**

Feline Respiratory Pathogen PCR Kit

**【Packing Specification】**

10 Tests/Box

**【Intended Use】**

This kit is used for the qualitative detection of the Feline Calicivirus (FCV), Feline Herpesvirus type I (FHV-1), Bordetella bronchiseptica (Bb), Mycoplasma felis (MF), Feline Chlamydia (CF) genes in samples, assisting in the diagnosis of infection.

**【Principles of Testing】**

This kit uses real-time fluorescence PCR technology, specific primers and fluorescent probes are designed targeting specific fragments of the FCV, FHV-1, Bb, MF, CF genes. An exogenous internal control is added to the reagent to monitor the entire detection process.

**【Product Composition】**

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Number	Component	Specification
1	Test reagent	10 portions (3 tubes/portion)
2	Sample processing solution	10 tubes (1.5 mL/tube)
3	Disposable sampling swab	10 pieces
4	Self-suction dropper	10 pieces
5	Conical tube	10 pieces

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**【Storage】**

1. Storage Conditions: 2°C~30°C, avoid light, and avoid heavy pressure.
2. Product Shelf Life: 24 months.

**【Testing Instrument】**

The real-time fluorescence quantitative PCR analyzer produced by Tianjin MNCHIP Technologies Co., Ltd.

**【Sample Requirements】**

The required sample usage is oral, nasal, and eye swabs.

**【Procedure】**

## 1. Sample Collection

(1) Take out the sample processing liquid and a conical tube. Invert the conical tube with the tip downwards, puncture the membrane of the sample processing liquid tube, and set aside for later use.

(2) Use a disposable sampling swab to gently collect secretions from the oral cavity, nasal cavity, or lower eyelid. Quickly place the swab into the sample processing solution tube and stir it adequately to ensure that the sample on the swab is fully dissolved in the solution. Break off the tail end of the swab.

## 2. Reagent Preparation

Take out the PCR reaction tube containing the detection reagent and set it aside for later use. Do not open the tube cap.

## 3. Sample addition.

(1) Sample aspiration<sup>※</sup>:

Take a self-suction dropper, handle it gently at the top without pressing the bulb. Insert the dropper below the liquid surface of the sample processing solution, until the black graduation line aligns with the liquid level. Hold for 3 seconds, then slowly withdraw. At this point, the self-suction dropper has automatically completed the sample aspiration.

**※ Approximately 20  $\mu$ l of sample volume should be aspirated, ensuring the liquid volume is not above or below 1/4 of the black graduation line of the pipette. A contamination-free pipettor can also be used to aspirate the sample (20  $\mu$ l).**

**※ If excess processing liquid is inadvertently aspirated, completely expel the liquid from the pipette before reaspirating.**

(2) Sample transfer<sup>※</sup>:

Open the PCR reaction tube cap, insert the self-suction dropper into the PCR reaction tube, and gently press the bulb at the top of the dropper to transfer all the liquid into the reaction tube. Close the cap tightly, vortex the tube twice for 10 seconds each time to ensure the reagent is fully dissolved. Gently flick the tube to remove any bubbles, then briefly centrifuge it.

Follow the above procedure to complete the transfer of samples to the remaining 2 PCR reaction tubes.

**※ Be careful to avoid dislodging the lyophilized bead inside the tube during the operation.**

#### 4. PCR Amplification

Place the 3 reaction tubes into a fluorescence quantitative PCR analyzer (please insert them into the corresponding wells as indicated on the analyzer interface) to perform PCR amplification.

#### 5. Sample Storage<sup>※</sup>

Store at 2-8°C for no more than 3 days or at -20°C for no more than 1 month, avoiding repeated freeze-thaw cycles.

※ When storing or discarding the sample, the tip of the conical tube can be removed from the tube and used as a stopper inserted into the sample processing solution tube to prevent large spills.

#### 【Result Interpretation】

Target Ct	Internal Control Ct	Result Interpretation
≤36	≤36	Positive
	>36 or NoCt <sup>*1</sup>	
>36 or NoCt	≤36	Negative
>36 or NoCt	>36 or NoCt	Suggest retesting <sup>*2</sup>

\*1: When the pathogen concentration in the sample is high, due to competitive relationships, the internal control may report negative.

\*2: When all target channel are negative, and the internal control result is also negative, it indicates strong inhibition or operational error in the sample. It is recommended to mix 200 μL of sample processing liquid with 400 μL of sterile water, then retest according to steps 3 and 4 of the operation procedure.

#### 【Limitations of Procedure】

1. Aerosol contamination of amplification products can lead to false positives, requiring segmented operations during testing experiments.
2. Negative results cannot completely rule out the possibility of pathogen infection; judgment should be made in conjunction with other clinical indicators.
3. The metabolic status of pathogens changes with the progression of the disease, leading to potential variations in the detection results of samples from different stages.

#### 【Performance Indicators】

1. Limit of Detection: FCV 1\*10<sup>3</sup> copies/mL, FHV-1 3\*10<sup>3</sup> copies/mL, MF 3\*10<sup>3</sup> copies/mL, CF 5\*10<sup>3</sup> copies/mL, Bb 3\*10<sup>3</sup> copies/mL
2. Precision: The same precision reference sample was tested over 5 days by 2 operators using 3

different reagent lots. The within-run and between-run precision, within-day and between-day precision, as well as within-operator and between-operator precision, all have coefficients of variation (CVs) not exceeding 5%.

3. Internal Positive Reference Sample Concordance Rate: The concordance rate for 10 positive reference samples is 100%.

4. Internal Negative Reference Sample Concordance Rate: The concordance rate for 10 negative reference samples is 100%.









5. Pecificity: There is no cross-reactivity with the pathogens listed in the table below, and no interference from common interfering substances.

<b>Cross-reactivity</b>	
Escherichia coli	Staphylococcus aureus
Streptococcus canis	Salmonella Typhimurium
<b>Interfering substances</b>	
Blood	Mucins

**【Notes】**

- Carefully read the instructions before starting the test and operate as required.
- Do not cross-use items between zones to avoid contamination caused by human factors.
- Errors in sample collection, storage, transportation conditions, and operational procedures may affect test results.
- Replace self-suction pipettes when drawing different samples.
- After adding samples, tightly cover the tube cap, thoroughly mix using a vortex mixer, gently tap the tube wall to remove air bubbles. Inadequate mixing may pose risks of false negatives or false positives.
- After the reaction is complete, the reaction tubes and other contaminants must be disposed of in accordance with local regulations.
- Do not use expired products; reagents from different batches should not be mixed or interchanged.

**【Symbols Used in Labelling】**

Symbol	Explanation
	Veterinary use only
	Manufacturer
	Use-by date
	Batch code
	Date of manufacture
	Consult instructions for use
	Limit of temperature
	Do not re-use

**【Manufacturer】**



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