

Canine Respiratory Disease Detection Kit

(Canine Distemper virus/Canine Adenovirus Type II/Canine parainfluenza virus/Bordetella bronchiseptica/Mycoplasma cynos/Influenza A Virus)
(PCR-Fluorescence Probe Method)

Instruction For Use
(REF : JVP055)

[Product Name]

English Name: Canine Respiratory Disease Detection Kit (Canine Distemper virus/Canine Adenovirus Type II/Canine parainfluenza virus/Bordetella bronchiseptica/Mycoplasma cynos/Influenza A Virus)
(PCR-Fluorescence Probe Method)

[Packing Specification]

8 T/Kit

[Intended Use]

The kit uses the fluorescent PCR method to detect the Canine Distemper virus/Canine Adenovirus Type II/Canine parainfluenza virus/Bordetella bronchiseptica/Mycoplasma cynos/Influenza A Virus Bordetella in the samples of the eye, nasal and throat swabs of pets. It is suitable for detection, diagnosis and epidemiological investigation for Six Canine Respiratory Tests.

[Testing Principle]

Nucleic acid (DNA/RNA) of samples is extracted with nucleic acid extraction reagent. Under the action of efficient reverse transcriptase, RNA is taken as the template, and the cDNA chain complementary to the RNA template is generated through synthesis reaction. Under the action of the Taq enzyme, DNA is taken as the template, and the copy number of the specific target fragments is amplified through the circulation of denaturation under high temperature and annealing and extension under medium temperature. Through hybridization of the specific probe with fluorescence labeling and extended target fragment and by making use of the activity of the 5'→3' exonuclease of Taq polymerase, the reporter gene and the quenching gene of the fluorescence probe area separated, and specific fluorescence signals are sent out. Fluorescence signals are detected with fluorescence PCR equipment. The result is judged based on the Ct value of the sample and the shape of the amplification curve.

[Kit Components and Specifications]

	Component	QTY
Nucleic Acid Extraction Reagent	Kit	8 pcs.
	Magnetic rod cover	8 pcs.
PCR Amplification Reagent	PCR freeze-dried reagent	8 pcs.
Disposable Sampling Kit	Swab	8 pcs.
	Sample preservation reagent	8 tubes
	Manual	1 pcs.

[Storage Condition and Validity]

The nucleic acid extraction reagent is stored under ambient temperature. PCR freeze-dried reagent should be stored under ambient temperature, without exposure to light. The kit is valid for 18 months with storage under 2-8°C. And the kit will be valid for 12 months with storage under 8~30°C. Please use it within the validity period.

[Operating Environment of the Instrument]

It is recommended to carry out the test at an ambient temperature of 20-25°C.

[Sample Type]

Eye,Nasal and throat swabs

[Sampling requirements]

Liquid sample: (including blood samples, ascites, pleural effusion)
Take 200μL directly and add it into the preservation reagent, shake and mix well for use. If the sample is excessive, too concentrated or thick, it must be diluted before test. Urine and CSF sample ,it is recommended to directly add 200μL into the LB tube for extraction.

Swab sample:

1. Fecal swab: Use the swab to dip the right amount;
2. Anal swab: Moisten the swab with diluent first, and then wipe the sample;
3. Nasopharyngeal swab: Apply a swab under the eyelid to collect the eye swab sample; use a swab to moderately wipe oral and nasal secretions, and collect the nasopharyngeal swab sample;
4. After the swab sample is collected, the swab head should be quickly broken into the preservation solution, and then shaken sufficiently to fully dissolve the pathogen on the swab head into the preservation solution. After the sample collection is completed, it is recommended to perform nucleic acid extraction immediately; if necessary, the sample can be temporarily stored at 4°C or stored for a long time below -20°C; when the sample is sent for inspection, it needs to be transported in cold storage.

[Preparation Before the Experiment]

1. Before the experiment, please carefully read this manual, get familiar with each step, and use this kit strictly according to the requirements in the manual.
2. During operation, the operator should wear disposable gloves and masks, and all operations shall be carried out under conditions that can meet the PCR detection experiment environment requirement.
3. Please take out the corresponding number of extraction reagent card boxes based on the quantity of samples. Please refer to the identification content on the lateral side of holes of the card.

[Operation Steps]

Nucleic acid extraction:

- 1 Remove the corresponding amount of extraction reagent kit and tear the top sealing film based on identification. Each hole position of the kit is as shown in the schematic diagram.



- 2 Take a 200μL sample with a pipette and add to the LB hole.
- 3 Take one magnetic rod cover and place in the magnetic rod cover position in the kit.
- 4 Press the "In/Out" button  on the equipment, place the kit on the kit carrier for automatic extraction, press the "In/Out" button again to make the kit enter the instrument and close the chamber door.

Note: The kit should be placed in the kit carrier along the direction identified on the lateral side and should be placed on the bottom evenly. If the kit is normally placed, the red indicator of the corresponding channel on the instrument panel will be on, otherwise the indicator will not be on.

- 5 Press the "Run" button  to make the equipment start to extract nucleic acid, wait for completion of running of the equipment and the buzzing sound and then press the "In/out" button again to take out the card box.

Note: It is suggested that extracted nucleic acid sample should be detected immediately. If the sample is not used temporarily, please store it under -20°C or -80°C.

PCR amplification:

1. Take 20μL nucleic acid supernatant extracted from the EB hole with the pipette, add it to the PCR reagent tube, flick the reagent tube with fingers to make the dry powder dissolve evenly, swing all the liquid in the tube to the bottom of the tube and then put the tube in the reaction well of the PCR instrument.

Note:

- a) When using a pipette to suck the nucleic acid solution, do not suck any bubbles that may exist in the solution;
- b) The freeze-dried reagent must be fully mixed, and all the reaction liquid in the tube must be swung to the bottom of the tube, otherwise the background interference signal is likely to be too strong, thus affecting the judgment of results.
2. Select the corresponding program of pet pathogen, set the sample and detection item information, and click the Run icon to start the reaction;
3. After the reaction is over, take out the PCR product test tube in time and pack it in a ziplock bag for unified disposal.

[Result Interpretation]

Hole No.	Detection Channel	Typical S-type Amplification Curve	Ct Value	Result
1	FAM	Yes	<36	Canine Distemper virus Positive
	VIC	Yes	<36	Canine Adenovirus Type II Positive
2	FAM	Yes	<36	Canine parainfluenza virus Positive
	VIC	Yes	<36	Bordetella bronchiseptica Positive
3	FAM	Yes	<36	Mycoplasma cynos Positive
	VIC	Yes	<36	Influenza A Virus Positive
1	FAM	No	0 or ≥36	Canine Distemper virus Negative
	VIC	No	0 or ≥36	Canine Adenovirus Type II Negative

Hole No.	Detection Channel	Typical S-type Amplification Curve	Ct Value	Result
2	FAM	Yes	<36	Canine parainfluenza virus Negative
	VIC	Yes	<36	Bordetella bronchiseptica Negative
3	FAM	Yes	<36	Mycoplasma cynos Negative
	VIC	Yes	<36	Influenza A Virus Negative

[Limitations of Detection Method]

1. The aerosol contamination of amplification products is easy to cause false positive, and thus the testing laboratory should be set up in strict accordance with the requirements of PCR testing laboratory;
2. Negative results cannot completely rule out the possibility of pathogen infection, which should be judged in combination with other clinical indexes.

[Product Performance Indexes]

1. Sensitivity: The detection limit of this kit is 1000 copies/ml.
2. Specificity: This kit does not have cross-reaction with samples such as parvovirus, coronavirus, feline immunodeficiency virus and feline leukemia virus.
3. Precision: A strong positive and a weakly positive specimen were repeatedly repeated 10 times, and the CV value of the Ct value was less than 5%.

[Precautions]

1. Product check: Before using PCR amplification reagents, first unpack to check the dry powder at the bottom of the tube is normal (white, agglomerated). If it has been liquefied, it cannot be used again, otherwise it will affect the PCR results.
2. Pipette use: When 20μL of nucleic acid supernatant is aspirated as an amplification template for PCR, ensure the pipette should only be pressed to the first gear, not to the bottom, otherwise the suction volume will exceed 20μL, affecting the detection results.

3. Sample information settings: Ensure that the PCR tube is placed at the same well as the sample set in the instrument. If the PCR tube is placed at the 1# well in the amplification chamber, select the corresponding 1# on the sample setting interface for settings. After the sample name and detection item are set, click the Run icon to start the PCR amplification reaction.

【Icon Illustration】

Lable	Meaning	Lable	Meaning
	Date of manufacture		Consult instructions for use
	Manufacturer		Do not re-use
	Batch code		Keep away from sunlight
	Temperature limit		Contains sufficient for <n>tests
	Use-by date		Catalogue number