

# Canine Neosporidium (Ne) Nucleic Acid Detection Kit (Fluorescent PCR Method)

**Product Number: DTK146**

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## Shipping and Storage

1.  $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$ , stored in the dark, transported, and subjected to repeated freeze-thaw cycles no more than 5 times, with a validity period of 12 months.
2. The collected or processed samples should be stored at  $2^{\circ}\text{C}\sim 8^{\circ}\text{C}$  for no more than 24 hours; If long-term storage is required, it should be stored at  $-70^{\circ}\text{C}$  or below, with no more than 3 freeze-thaw cycles.

## Component

Component	50T
Ne reaction solution	500 $\mu\text{L}\times 2$
Enzyme solution	50 $\mu\text{L}$
Ne positive quality control product	50 $\mu\text{L}$
Negative quality control product	250 $\mu\text{L}$

**Note: Different batches of reagents cannot be mixed.**

## Description

This kit uses TaqMan probe method for real-time fluorescence PCR technology, designs a pair of canine Neosporidium specific primers, combines with a specific probe, and uses fluorescence PCR technology to amplify and detect the DNA of canine Neosporidium in vitro, which is used for pathogenic diagnosis of suspected infectious materials in clinical practice.

## Application

This reagent kit is suitable for detecting canine Neosporidium in animal feces and other samples, and is used for auxiliary diagnosis of canine Neosporidium infection.

## Applicable instruments

ABI7500, Agilent MX3000P/3005P, LightCycler, Bio-Rad, Eppendorf and other series of fluorescence quantitative PCR detectors.

## Specimen collection

Dead or euthanized animals, sterile collection of samples from lung lesions or the junction of lesions/non lesions; Use sterilized cotton swabs to collect tracheal secretions and place them in sterile test tubes.

## Protocol

### 1. Sample processing (sample processing area)

#### 1.1. Sample pre-processing

Organizational samples: Weigh approximately 1g of each tissue from 3 different positions, cut and mix it with surgical scissors, and then take 0.5g and grind it in a grinder. Add 1.5mL of physiological saline and continue grinding. After homogenization, transfer it to a 1.5mL sterile centrifuge tube and centrifuge at 8000rpm for 2 minutes. Take 100 $\mu\text{L}$  of supernatant and transfer it to a 1.5mL sterile centrifuge tube; Take 100 $\mu\text{L}$  of throat swab sample directly into a 1.5mL sterilized centrifuge tube.

#### 1.2. Nucleic acid extraction

**For Research Use Only**

We recommend using our company's nucleic acid extraction or purification reagents (magnetic bead method or centrifugal column method) for nucleic acid extraction. Please follow the reagent instructions for operation.

## 2. Reagent preparation (reagent preparation area)

Based on the total number of samples to be tested, the required number of PCR reaction tubes is N (N=number of samples+1 negative control tube+1 positive control tube); For every 10 samples, an additional 1 sample is prepared. The preparation of each test reaction system is shown in the table below:

reagent	Ne reaction solution	Enzyme solution
Dosage (sample size N)	19 $\mu$ L	1 $\mu$ L

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 20 $\mu$ L per tube.

## 3. Sample addition (sample processing area)

Take 5 $\mu$ L of the nucleic acid, positive control sample, and negative control sample extracted in step 1, and add them to the corresponding reaction tubes. Cover the tubes, mix well, and briefly centrifuge.

## 4. PCR amplification (nucleic acid amplification zone)

4.1. Place the reaction tube to be tested in the reaction tank of the fluorescence quantitative PCR instrument;

4.2. Set the channel and sample information, and set the reaction system to 25 $\mu$ L;

Fluorescence channel selection: Detection channel (Reporter Dye) FAM, Quencher Dye NONE, please do not select ROX reference fluorescence for ABI series instruments, select None.

4.3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	Collect fluorescence signals
1	1 cycle	95°C	2min	No
2	45 cycles	95°C	15sec	No
		60°C	30sec	Yes

## 5. Result analysis and judgment

### 5.1. Result Analysis Condition Setting

(Please refer to the user manuals of each instrument for setting up, taking the ABI7500 instrument as an example)

After the reaction is complete, the results will be automatically saved. Based on the analyzed image, adjust the Start value, End value, and Threshold value of the baseline (users can adjust them according to their actual situation, with Start value set between 3-15 and End value set between 5-20, so that the threshold line is in the exponential period of the amplification curve, and the amplification curve of negative quality control products is flat or below the threshold line). Click Analyze to automatically obtain the analysis results.

### 5.2. Result judgment

Positive: The Ct value of the detection channel is  $\leq 40$ , and the curve shows a significant exponential growth curve;

Negative: The Ct value of the sample test result is greater than 40 or there is no Ct value.

## Quality control standards

1. Negative quality control product: no specific amplification curve or Ct value display;
2. Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is  $\leq 32$ ;
3. The above conditions should be met simultaneously, otherwise the experiment will be considered invalid.

## Limitations of detection methods

1. The results of sample testing are related to the quality of sample collection, processing, transportation, and preservation;
2. Failure to control cross contamination during sample extraction can result in false positive results;
3. Leakage of positive controls and amplification products can lead to false positive results;
4. Genetic mutations and recombination of pathogens during epidemics can lead to false negative results;
5. Different extraction methods have differences in extraction efficiency, which can lead to false negative results;
6. Improper transportation, storage, or preparation of reagents can lead to a decrease in reagent detection efficiency, resulting in false negatives or inaccurate quantitative testing results;



## **MEBEP TECH(HK) Co., Limited**

Email: [sales@mebep.com](mailto:sales@mebep.com) Website: [www.mebep.com](http://www.mebep.com)

Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

7. The test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.

### **Note**

1. All operations must be strictly carried out in accordance with the instructions;
2. The various components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged before use;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction, and cover the tube tightly;
5. Use disposable suction tips, disposable gloves, and specialized work clothes for each area;
6. Sample processing, reagent preparation, and sample addition should be carried out in different areas to avoid cross contamination;
7. After the experiment is completed, treat the workbench and pipette with 10% hypochlorous acid, 75% alcohol, or a UV lamp;
8. All items in the reagent box should be treated as contaminants and disposed of in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".