

# Leptospira spp. (Lep) Nucleic Acid Detection Kit (Fluorescent PCR Method)

**Product Number: DTK314**

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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                       |
|--------------------------------------|---------------------------|
| Lep reaction solution                | 500 $\mu\text{L}\times 2$ |
| Enzyme solution                      | 50 $\mu\text{L}$          |
| Lep 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 specific primers and probes designed for the conserved region of the Leptospira gene, and employs fluorescence PCR technology to amplify and detect the nucleic acid of Leptospira in vitro, for clinical pathogen diagnosis of suspected infectious materials.

## Application

Leptospirosis is a natural zoonotic infectious disease caused by pathogenic Leptospira. Its clinical symptoms are diverse, mainly including fever, jaundice, hemoglobinuria, hemorrhagic mass, miscarriage, skin and mucosal necrosis, edema, etc. There are a total of 18 serogroups and 74 serotypes in China, widely present in species such as mammals and reptiles. The most closely related sources of infection to humans are mice, pigs, cows, and dogs. Rodents mainly carry jaundice hemorrhagic groups, while pigs and dogs mainly carry Pomona groups and dog groups, respectively. The clinical symptoms of leptospirosis are complex and easily misdiagnosed in the early stages, and the bacterial culture method poses safety hazards; Antibody testing has poor specificity and is prone to false positive results. PCR technology amplifies specific fragments on the genome of Leptospira, which can quickly and specifically screen and diagnose leptospirosis [2-3].

This kit is suitable for detecting Leptospira in animal organs, blood and other samples, and is used for auxiliary diagnosis of Leptospira infection.

## Applicable instruments

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

## Specimen collection

1g of tissues such as heart, liver, spleen, lungs, kidneys, lymph nodes, etc. were collected from dead animals; Extract 3-5mL of blood into EDTA-2Na anticoagulant tubes using sterile syringes.

## Protocol

**For Research Use Only**

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

**1.1. Sample Preparation**

Weigh approximately 1g of each tissue from three different locations, 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 100μL of the supernatant and transfer it to a 1.5mL sterile centrifuge tube; Blood sample: Take 50μL of anticoagulant lower layer blood cells, add 100μL of double distilled water and blow evenly.

**1.2. Nucleic acid extraction**

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

**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 following table:

| reagent                | Lep Reaction solution | Enzyme solution |
|------------------------|-----------------------|-----------------|
| Dosage (sample size N) | 19μL                  | 1μL             |

Transfer the mixed test reaction solution into a PCR reaction tube at a concentration of 20μL per tube.

**3. Sample addition (sample processing area)**

Take 5μL 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 25μL;

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 ≤ 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**

Negative quality control product: no specific amplification curve or Ct value display;

Positive quality control product: The amplification curve shows a significant exponential growth period, and the Ct value is ≤32;

The above conditions should be met simultaneously, otherwise the experiment will be considered invalid.

**Limitations of detection methods**



## **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

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. During the epidemic, genetic mutations and recombination of pathogens 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 inaccurate preparation of reagents can lead to a decrease in reagent detection efficiency, resulting in false negatives or inaccurate quantitative testing results;
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 kit should be treated as contaminants and handled in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".