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Toxoplasma (TOX) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK082

Shipping and Storage

1. -20℃±5℃, 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℃~8℃ for no more than 24 hours; If long-term storage is required, it should be stored at -70℃ or below, with no more than 3 freeze-thaw cycles.

Component

Component	50T
TOX reaction solution	500μL×2
Enzyme solution	50μL
TOX positive quality control product	250μL
Negative quality control product	250μL

Note: Different batches of reagents cannot be mixed.

Description

This kit employs primers and probes specifically designed for the Toxoplasma gondii genome, utilizing fluorescence PCR technology to amplify and detect nuclear DNA in vitro, enabling clinical pathogen diagnosis of suspected infected samples.

Application

Toxoplasmosis is a zoonotic disease caused by Toxoplasma gondii, with humans and animals as intermediate hosts and cats as its ultimate host. This disease is widely distributed around the world and seriously endangers the health of humans and animals. China has classified it as a Class II animal disease. Toxoplasma gondii often causes animal fever, dyspnea, neurological symptoms and extreme weakness, and a little cough, nasal secretions, foam vomit at the mouth, shaking, shaking head, dehydration and diarrhea. When female animals get sick, it causes abortion, causing great losses to animal husbandry everywhere. At present, there is no ideal drug or vaccine for treating Toxoplasma gondii. Timely diagnosis and prevention are effective measures to control the disease. Real time fluorescence PCR technology has become the development trend for detecting toxoplasmosis due to its high sensitivity, high specificity, and timely detection.

This kit is suitable for detecting Toxoplasma gondii in blood, cerebrospinal fluid, liver, spleen, kidney, suspicious food and other samples, and is used for auxiliary diagnosis of Toxoplasma gondii infection.

Applicable instruments

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

Specimen collection

Extract 3-5mL of blood using a sterile syringe into an EDTA-2Na anticoagulant tube; For animals or patients with neurological symptoms, cerebrospinal fluid should be collected for testing; Take 1g of suspected contaminated food for testing.

Protocol

1. Sample processing (sample processing area)

1.1. Sample pre-processing

Solid sample: After surgical cutting and mixing, 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 supernatant and transfer it to a 1.5mL sterile centrifuge tube; Liquid sample: Take 100μL directly into a 1.5mL sterilized centrifuge tube; Take 100μL of cerebrospinal fluid directly into a 1.5mL sterilized centrifuge tube; Blood sample: Take 50μL of middle layer white blood cells by anticoagulant centrifugation, add 100μL of double distilled water and blow well.

1.2. Nucleic acid extraction

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	TOX 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 20uL 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℃	2min	No
2	45 cycles	95℃	15sec	No
		60℃	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

Negative: The sample test result shows no Ct value and no specific amplification curve.

Suspicious: If the sample test result is 40<Ct value ≤ 45, it is recommended to repeat the test. If the detection channel is still 40<Ct value ≤ 45 and the curve has a clear exponential growth curve, it is judged as positive. Otherwise, it is judged as negative.

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 ≤ 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;
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 laboratory should be treated as pollutants and handled in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".