

## **Anisakis spp. (Ani) Nucleic Acid Detection Kit (Fluorescent PCR Method)**

**Product Number: DTK317**

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### **Shipping and Storage**

Low temperature transportation, stored at -20°C, with a shelf life of 12 months.

### **Component**

Component	96T
2×Taq PCR Master Mix	600μL
DEPC-H <sub>2</sub> O	1mL
PCR Primer Mixture of Heterozygous Nematodes	60μL
PCR positive control of Heterobranchia genus (1 × 10E5 copies/μL)	50μL

Note: To avoid the spread of infectious pathogens, this product does not provide live samples as positive controls, only specific nucleic acid fragments are provided as positive controls

### **Description**

This reagent kit can be used to detect the genus Heterobranchia. The genus Heterobranchia is a parasitic worm belonging to nematodes, with a white body and a slightly dull end. Its life cycle is completed through fish and mammals in the ocean. Although it cannot complete its life cycle through human development and maturity, ingestion of fish containing larvae or undercooked meat can cause infection in diners, and patients may experience severe abdominal pain or allergic reactions.

### **Application**

This product is a specialized kit developed based on the PCR principle for detecting the genus Nematodes. It has the following characteristics:

1. Ready to use, users only need to provide a sample DNA template.
2. Provide a positive control to distinguish false negative samples.
3. Design primers based on specific sequences to specifically detect the genus Heterodonia.
4. The PCR mix contains sample dyes, which can be directly loaded for electrophoresis after PCR.
5. This product is sufficient for 50 PCR reactions in a 20μL system.
6. This product can only be used for scientific research.

### **Specimen collection**

Sample DNA

### **Protocol**

#### **1. DNA extraction (sample preparation area)**

This kit is compatible with most DNA extraction kits on the market for extracting and purifying DNA samples using a self selected method.

We recommend using our company's DNA extraction kit to extract samples.

#### **2. Reagent Preparation (Reagent Preparation Area)**

If there are N samples to be tested, prepare N+2 qPCR tubes (N samples to be tested+1 negative control+1 positive control) and add the following components to each qPCR tube.

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component	Sample tube N	qPCR	qPCR
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**For Research Use Only**

		negative control	positive control
2×Taq PCR Master Mix	10μL	10μL	10μL
PCR Primer Mixture of Heterozygous Nematodes	1μL	1μL	1μL
DEPC-H <sub>2</sub> O	7μL	7μL	7μL

**Transfer to the sample preparation area.**

### 3. Add Template (Template Add Area)

Add 2μL of template to the PCR tube, in the order of negative control (DEPC-H<sub>2</sub>O), sample template to be tested, and PCR positive control for the genus Nematodes. Centrifuge for 30 seconds and immediately perform amplification reaction.

### 4. Amplification reaction (amplification and product analysis area)

Place the PCR tube in the corresponding position of the sample slot of the PCR amplification instrument for amplification. The amplification procedure is as follows:

Step	Temperature	Time
Pre denaturation	95°C	3min
qPCR reaction	95°C	15sec
(45 cycles)	55°C	15sec
	72°C	30sec
Extend	72°C	5min

### 5. Electrophoresis analysis

- 5.1. Take 5-10μL of PCR product. Electrophoretic detection of PCR products. The 2 × Taq PCR Master Mix provided by this product contains dyes and can be directly loaded after amplification without the need for additional loading buffer.
- 5.2. Positive control result: There is a 952bp band.
- 5.3. Negative control result: No 952bp band.
- 5.4. Sample testing results: There is a band of 800-1100bp, indicating that the sample belongs to the genus Heterobranchia, and the result is positive; There is no 800-1100bp band, indicating that the sample is not of the genus Heterobranchia, and the result is negative.