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Ovine Theileria Dye-Based Quantitative PCR Kit

Product Number: DTK567

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of one year. Positive controls need to be placed separately and should not contaminate other reagents.

Component

Component	50T	Nine hole box packaging
2 ×SYBR qPCR Mix	550μL	White cover
DEPC-H_2O	1mL	Yellow cover
Fluorescent template diluent	1mL	Orange cover
Sheep Taylor worm dye method qPCR primer mixture	260μL	Blue cover
Sheep Taylor worm dye qPCR positive control (1 × 10E8 copy/μL)	50μL	Red cover

Description

Ovine Theileriosis is a blood borne protozoan disease in which Ovine Theileriosis parasitizes macrophages, lymphocytes, or red blood cells in goats or sheep. At present, various Taylor worms that can infect sheep have been discovered.

Application

This product is a sheep Taylor worm detection kit developed based on the principle of dye based fluorescence quantitative PCR. It can detect T. lestoquarti, T. ovis, T. recondita, T. luwenshuni, T. uilenbergi, T. annulata, T. equi, and T. orientalis. It has the following characteristics:

1. Ready to use, users only need to provide a sample DNA template.
2. Primers and other components have been optimized for high sensitivity.
3. Provide positive controls to distinguish false negative samples.
4. High specificity, primers are designed based on highly conserved regions of sheep Taylor worm DNA, and will not cross react with DNA from other organisms.
5. It can be used for both qualitative and quantitative testing. When used for quantitative detection, the linear range should be at least 5 orders of magnitude.
6. This product is sufficient for 50 dye based fluorescent quantitative PCR reactions in a 20μL system.
7. This product can only be used for scientific research.

Protocol

1. DNA extraction (sample preparation area)

1.1. If there are N samples to be extracted, it is best to set N+2 extractions, with the additional being PC (positive control for sample preparation) and NC (negative control for sample preparation). You can take 10μL of 1000 fold dilution of the positive control and add a certain amount of water to make the total volume consistent with the specified volume of the sample to be extracted, and use it as PC. Additionally, use water as NC.

1.2. Extract and purify sample DNA using a self selected method, and this kit is compatible with most nucleic acid extraction kits on the market. We recommend using our company's DNA extraction kit for extraction.

2. Dilute standard curve sample (sample preparation area)

Due to the high concentration of positive control, the following dilution operations must be performed in a separate area to avoid contaminating the sample or other components of this kit.

2.1. Mark 6 centrifuge tubes, namely 7, 6, 5, 4, 3, and 2.

2.2. Add 45μL of fluorescent template diluent separately using a core gun tip (preferably using a core gun tip, the same below).

2.3. Add 5μL of 1 × 10E8 copy/μL positive control (provided by the reagent kit) to tube 7, shake thoroughly for 1 minute, and obtain 1 × 10E7 copy/μL standard curve sample. Put it on ice for later use.

2.4. Change the gun head and add 5μL of 1 × 10E7 copy/μL positive control (obtained from the previous dilution) to tube 6. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E6 copy/μL. Put it on ice for later use.

2.5. Change the gun head and add 5μL of 1 × 10E6 copy/μL positive control (obtained from the previous dilution) to tube 5. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E5 copy/μL. Put it on ice for later use.

2.6. Repeat the above operation until obtaining standard curve samples with 6 dilutions.

Put it on ice for later use. If no standard curve is required, dilute the positive control to 1 × 10E5 copies/μL.

3. Reagent preparation (reagent preparation area)

Add the following components to each qPCR tube:

Component	N sample tubes to be tested	qPCR negative control	qPCR positive control
2 ×SYBR qPCR Mix	10μL each	10μL	10μL
50× ROX Reference Dye I or II (User provided)	0.4μL each	0.4μL	0.4μL
Sheep Taylor worm dye method qPCR primer mixture	4.6μL each	4.6μL	4.6μL

Note: Models that require the use of ROX Reference Dye I: ABI Prism5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, Step-One, Step-One Plus.

Models that require the use of ROX Reference Dye II: ABI Prism 7500/7500Fast, ViiA7, Stratagene MX4000/MX3005P/MX3000P.

Models that do not require the use of ROX: Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4; Cepheid SmartCycler; Eppendorf Mastercycler ep realplex, realplex 2s; Illumina Eco qPCR; Qiagen/Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; Roche Applied Science LightCycler 480; Transfer the Thermo Scientific PikoReal Cycler (supplemented with DEPC-H_2O) to the sample preparation area.

4. Add Template (Template Add Area)

Add 5μL of template to each qPCR tube, in the order of negative control (DEPC-H_2O), test sample template, and sheep Taylor worm qPCR positive control. Centrifuge for 30 seconds and immediately perform amplification reaction.

5. Amplification reaction (amplification and product analysis area)

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

Process	Temperature	Time
Pre denaturation	95℃	3min
qPCR reaction (45 cycles)	95℃	20sec
	58℃	20sec
	72℃	20sec(SYBR channel collects fluorescence signals)
Perform melting curve analysis according to the preset program of the instrument		

6. Result analysis

6.1. If creating a standard curve, plot the standard curve with the log value of positive control concentration as the horizontal axis and Ct value as the vertical axis. Calculate the log value of the DNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and determine its concentration.

6.2. If no standard curve has been created, the results shall be judged according to the following criteria:

Positive control (1×10E5 copies/μL) result: Ct value<30, with significant exponential growth, showing a typical S-shaped

curve.

Negative control result: Ct value>40 or no Ct value, no significant exponential growth period or plateau period.

Sample testing results: Ct value<38, with a significant exponential increase, indicating the detection of sheep Taylor worm in the sample, and the result is positive; A Ct value greater than 40 or no Ct value indicates that no sheep Taylor worm was detected in the sample, and the result is negative; If the Ct value is within the range of 38-40, the sample should be retested. If the Ct value of the repeated experiment is still within the range of 38-40 and the peak of the melting curve is the same as the positive control, then the sample is judged as positive; If they are not the same, the sample is judged as negative.