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Porcine Proliferative Enteritis Dye-Based Quantitative PCR Kit

Product Number: DTK571

Shipping and Storage

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

Component

Component	50T	Nine hole box packaging
2×SYBR qPCR magic mix	500μL	Brown cover
Fluorescent PCR specific template diluent	1mL	Red cover
Ultrapure water	1mL	Green cover
Dye based qPCR primer mixture for porcine proliferative enteritis	100μL	White cover
Porcine proliferative enteritis dye based qPCR positive control (1 × 10E7 copy/μL)	50μL	Yellow cover

Description

Porcine proliferative enteritis is a common syndrome in pigs caused by a bacterium called intracellular bacteria. Fluorescent quantitative PCR is the mainstream technology for detecting infectious diseases.

Application

This product is a specialized kit developed based on dye based fluorescence quantitative PCR technology for detecting porcine proliferative enteritis. 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, the primers are designed based on the highly conserved region of porcine proliferative enteritis DNA and will not cross react with the DNA of other microorganisms.
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.

Specimen collection

Sample DNA.

Protocol

1. Dilute standard curve samples (taking the 10 fold dilution of 6 copies/μL of 10E1-10E6 as an example)

Due to the high concentration of the standard substance, the following dilution operations must be carried out in a separate area and must not contaminate the sample or other components of this kit. To increase product stability and avoid the spread of infectious pathogens, this product does not provide live samples as positive controls, only non infectious DNA fragments are provided as positive controls.

1.1. Mark 6 centrifuge tubes, namely 6, 5, 4, 3, 2, 1.

1.2. Add 45μL of fluorescent PCR specific template dilution solution using a core gun tip, preferably using a core gun tip. The same applies below.

1.3. Add 5μL of 1 × 10E7 copy/μL positive control (provided by the reagent kit) to tube 6, shake thoroughly for 1 minute, and obtain 1 × 10E6 copy/μL standard curve sample. Put it on ice for later use.

1.4. 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.

1.5. Change the gun head and add 5μL of 1 × 10E5 copies/μL positive control (obtained from the previous dilution) to tube 4. Shake thoroughly for 1 minute to obtain a standard curve sample of 1 × 10E4 copies/μL. Put it on ice for later use.

1.6. Repeat the above operation until obtaining standard curve samples with 6 dilutions. Put it on ice for later use.

2. Preparation of Sample RNA

2.1. 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 a 10000 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, which can be used as PC. Additionally, use water as NC.

2.2. This kit is compatible with most DNA extraction kits on the market for purifying sample DNA using a self selected method. Recommend using our company's DNA extraction kit for extraction.

3. SYBR qPCR reaction (20μL system, performed in the sample preparation room)

3.1. If quantitative analysis is performed and only one repetition is made, label N+9 PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for PCR negative control (using water as a template), and 6 are used for the standard curve. If qualitative analysis is performed and only one repetition is made, label N+4 PCR tubes, of which N+2 are used for the N+2 samples obtained in the previous step, 1 is used for PCR negative control (using water as a template), and 1 is used for PCR positive control (using the positive control dilution of tube 4 as a template). Below, only quantitative analysis will be used as an example to describe the operational steps.

3.2. Add each component to the labeled tube according to the table below (this table only lists one repetition. The positive control is only set after the sample tube and negative control are set, and the positive control sample is added after all tubes are covered and stored):

Component	Sample tube N+2	RT-PCR negative control tube	RT-PCR positive control (1-6 tubes)
2×SYBR qPCR MagicMix	10μL	10μL	10μL
Dye based qPCR primer mixture for porcine proliferative enteritis	2μL	2μL	2μL
N+2 DNA templates to be tested	8μL	-	-
Ultrapure water	-	8μL	-
Step 6: Standard Curve Sample Dilution Solution (1-6)	-	-	12μL (sample 1 to tube 1, sample 2 to tube 2...)

3.3. Cover the machine with the lid and perform PCR according to the following parameters:

Process	Temperature	Time
Pre denaturation	95℃	10 min
qPCR reaction (40 cycles)	95℃	15sec
	60℃	60 sec (Collect fluorescence signals from SYBR channel)
Perform melting curve analysis according to the preset program of the instrument		

4. Data processing

4.1. If this reagent kit is used for quantitative detection, plot a 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 RNA concentration of the sample from the standard curve based on the Ct value of the sample to be tested, and then calculate its concentration.

4.2. If this kit is used for qualitative testing and only determines positive or negative, the negative control Ct must be greater than 40. The positive control must have fluorescence logarithmic growth, typical amplification curve, and Ct value

should be less than 35. For the test sample, if its Ct is ≥ 40, it is negative; if it is ≤ 35, it is positive. If it is between 35-40, repeat once. If the Ct value of the repeated experiment is ≥ 40, it is negative; if it is less than 40, it is positive. The melting curve must also be considered. If the melting Tm value differs from the target amplification fragment Tm value by ≥ 2 ℃, it is non-specific amplification and not true positive amplification.