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Paenibacillus mucilaginosus Probe-Based qPCR Kit

Product Number: DTK555

Shipping and Storage

Low temperature transportation, stored at -20℃, with a shelf life of 24 months.

Component

Component	Specification	Nine hole box packaging
2×Probe qPCR MagicMix	0.5mL	Natural color tube
Fluorescent PCR specific template diluent	1mL	Green cover
Ultrapure water	1mL	Blue cover
Gel like Bacillus subtilis qPCR Primer Probe Dry Powder	50	Brown cover
qPCR of gelatinous Bacillus subtilis Positive control (1E7 copy/μL)	50μL	Yellow cover

Note: Primer probe dry powder needs to be briefly centrifuged before use, and then 165μL of ultrapure water should be added to the centrifuge tube and thoroughly mixed before use. Unused samples need to be stored at -20℃.

Description

Paenibacillus mucilaginosus is a beneficial microorganism and an important functional bacterium in soil. It has the advantages of improving soil fertility, promoting plant growth, and improving soil structure. In agricultural production, gelatinous Bacillus subtilis is often used in the research and application of microbial fertilizers, which has a certain positive effect on improving crop yield and quality. Therefore, rapid detection of gelatinous Bacillus subtilis is of great significance.

Application

Our company has developed a detection kit for gel like Bacillus subtilis based on qPCR technology, which has the following characteristics:

1. Ready to use, users only need to provide a sample DNA template.
2. Primers and probes have been optimized for high analytical sensitivity, reaching up to 100 copies per reaction.
3. Provide positive controls to distinguish false negative samples.
4. High specificity, primers are designed based on highly conserved regions of Bacillus subtilis DNA, and will not cross react with DNA from other organisms.
5. It can be used for both qualitative and quantitative testing. The linear range for quantitative detection should be at least 5 orders of magnitude.
6. This product is sufficient for 50 qPCR reactions using a 20μL probe system.
7. This product can only be used for scientific research.

Specimen collection

Sample DNA.

Protocol

1. **Dilute the standard curve sample (taking the 10 fold dilution of 1E1-1E6 copies/μ L 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, and 1.

1.2. Add 45μL of fluorescent PCR specific template diluent with a core gun tip, preferably using a core gun tip, the same 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 (diluted in the previous step) 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 DNA

2.1. If there are N samples, it is best to set N+2 extractions, with the extra one being PC (positive control for sample preparation) and one being NC (negative control for sample preparation). You can use 10μL of the fourth dilution obtained in the previous step and add a certain amount of water to make the total volume equal to the required starting sample volume for each sample preparation, and use this 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. We recommend using our company's DNA extraction kit for extraction.

3. Probe 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 (directly using the positive control dilution of tube 4 in step 6 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 should be added after all tubes are covered and stored):

Component	Sample tube N+2	PCR negative control tube	Standard curve sample tube (1-6 tubes)
2×Probe qPCR MasterMix	10μL	10μL	10μL
qPCR Primer Probe Mixture of Colloidal Bacillus subtilis	3μL	3μL	3μL
N+2 DNA samples to be tested	7μL	-	-
Ultrapure water	-	7μL	-
Step 6: Standard Curve Sample Dilution Solution (1-6)	-	-	7μL (sample 1 to tube 1, sample 2 to tube 2...)

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

Process	Temperature	Time
Pre denaturation	95℃	10 min
qPCR reaction (40 cycles)	95℃	15sec
	60℃	60 seconds (fluorescence signal collected from FAM channel, quenching group TAMRA)

4. Data processing

4.1. If the result of sample preparation positive control or PCR positive control (including standard curve samples) is negative, the entire experiment is invalid, and data analysis is not required. A new sample preparation, PCR amplification, or contact with the manufacturer is needed. If the sample preparation negative control or PCR negative control result is

positive, it indicates environmental pollution, and the entire experiment is invalid. Data analysis is not required, and the manufacturer needs to be contacted.

4.2. If both negative and positive controls are normal, the experiment is valid and can proceed to subsequent analysis.

4.3. 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 DNA concentration of the sample from the Ct value of the test sample on the standard curve, and then calculate its concentration.

4.4. If this kit is used for qualitative testing and only determines positive or negative, the negative control Ct must not have a reading or be greater than or equal to 40. The positive control must have fluorescence logarithmic growth, typical amplification curve, and Ct value should be less than 40. If the Ct of the test sample is not read and is greater than or equal to 40, it is considered negative. If it is less than 40, it is considered positive.