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Listeria monocytogenes Nucleic Acid Detection Kit (Isothermal Fluorescent Method)

Product Number: DTK551

Shipping and Storage

Please store at -20℃ with a validity period of 12 months.

Component

Component	Content
A-LM-I	1200μL
B-I	55μL
C-I	1200μL
Positive control	50μL

Description

The pathogenic bacteria detection series produced by our company is based on unique constant temperature fluorescence detection technology, which can amplify specific nucleic acid fragments of pathogenic microorganisms in food, feed and other samples. The instrument monitors the fluorescence signal changes in real time during the amplification process and automatically interprets the results.

Application

This product is used for the detection of Listeria monocytogenes. The detection limit is 103 CFU/mL.

Applicable instruments

Dhelix3210, Dhelix-1610, Dhelix-Q5, ESETubeScanner, GenieII, Deaou-308C and other constant temperature fluorescence detection instruments, Gentier32R, Gentier48E/48R, CFX96, ABI7500, LightCycler480 and other fluorescence PCR instruments.

Consumables and instruments

Sterilize 1.5mL or 2.0mL centrifuge tubes; Sterilize 0.2mLPCR tube or eight tube; Ice box; Pipette (0.5-10μL, 10-100μL, 100-1000μL) and matching sterilization pipette tip; Centrifuge; Vortex mixer; metal bath.

Specimen collection

According to section 5.1 of the "GB4789.30-2010 National Food Safety Standard for Microbiological Examination of Food - Detection of Listeria monocytogenes", the samples were pre cultured and the prepared bacterial solution was stored for future use.

Take 25g (mL) of sample using aseptic operation and add it to a homogenization bag containing 225mL of LB1 enrichment solution. Homogenize continuously on a tapping homogenizer for 1-2 minutes; Alternatively, place it in a homogenized cup containing 225mL of LB1 enrichment solution and homogenize at 8000r/min to 10000r/min for 1-2 minutes. Cultivate at 30℃± 1℃ for 24 hours. Please follow the standard operation for detailed steps.

Protocol

Thaw the reagents completely and separate the hearts of each group for 30 seconds.

1. **Reagent preparation (reagent preparation area, placed in an ice box for preparation)**

If there are N samples to be tested, refer to the table below and calculate the dosage of each component based on N+2 quantities (N samples to be tested+1 negative control+1 positive control). Place the reaction solution in a 0.6mL or 1.5mL centrifuge tube,

vortex and mix well, centrifuge for 30 seconds, and divide into 0.2ml PCR tubes. Add 1 drop of C-I (about 20μL) to each tube.

Reagent	Usage
A-LM-I	22×(N+2)μL
B-I	1×(N+2)μL
Total volume of reaction solution	23×(N+2)μL

2. Template preparation (sample preparation area)

Suggest using reagents to complement the bacterial DNA extraction series products. Please refer to the product manual for specific procedures.

3. Add template (sample preparation area, placed on an ice box)

Add 2μL of template to the PCR tube containing the reaction solution in step 1, in the order of test sample template, positive control (negative control tube does not require additional template), centrifuge for 30 seconds, and immediately perform amplification reaction.

4. Amplification reaction (amplification and product analysis area)

4.1. React at 63℃ for 45 minutes using a constant temperature instrument. After the instrument is heated to 63℃, create a new program, set the experiment name and reaction time, place the PCR reaction tube centrifuged in step 3 into the constant temperature fluorescence molecular detector, and click start detection.

4.2. If using a fluorescence quantitative PCR instrument, select FAM as the fluorescent group and None as the quenching group. Use 63℃ 15s and 63℃ 45s as one cycle, and collect the fluorescence signal at 63℃ 45s for 45 cycles.

Please refer to the instrument manual for setting up other instruments.

5. Result analysis and judgment

5.1. The instrument automatically determines the result. If it shows "positive", the sample contains Listeria monocytogenes; If it shows' negative ', the sample does not contain Listeria monocytogenes or the content is below the detection limit.

5.2. On a fluorescence quantitative PCR instrument, determine the result based on the presence or absence of an "S" - shaped amplification curve. If there is an "S" - shaped amplification curve, the sample contains Listeria monocytogenes; If there is no "S" type amplification curve, the sample does not contain Listeria monocytogenes or the content is below the detection limit.

Note:The result of the negative control reaction tube shows "negative", and the result of the positive control reaction tube shows "positive". This test result is valid, otherwise it is invalid. If the repeated test results are still invalid, please contact the technical support personnel.

Note

1. This reagent has high detection sensitivity. To prevent pollution, the experiment needs to be conducted in different zones.
   1.1. Zone 1: Reagent preparation area.
   1.2. Second zone: Sample preparation zone.
   1.3. Third zone: amplification and product analysis zone.

   It is best to implement physical isolation between partitions to avoid pollution caused by human factors.
2. Wear work clothes and latex gloves during the experiment, use tools independently in different areas, and change gloves and lab clothes.
3. Strictly follow the operating steps. Please strictly follow the instructions for reagent preparation and sample addition on the ice box
4. The components in the reaction solution are sensitive to light and should be stored away from light. The reagent should be completely thawed before use, but repeated freezing and thawing should be avoided. It is recommended to centrifuge for 30 seconds before use and store the reaction solution in appropriate volumes according to the testing frequency.
5. After the reaction is complete, please dispose of the amplification tube in a sealed bag and clean it on the same day. Opening the lid may cause aerosol contamination and is prohibited.
6. Do not mix different batches of reagents and use them within their expiration date.

7. The detection limit is 103CFU/mL, and the bacterial genomic DNA extracted from the collected bacterial cells after centrifugation with 1mL of 103CFU/mL enrichment solution is used as a template.