

Salmonella universal probe fluorescent quantitative qPCR kit

Product Number: DTK249

Shipping and Storage

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

Component

Component	Specification	Nine hole box packaging
2×Probe qPCR MasterMix	500μL	Natural color tube
Fluorescent PCR specific template diluent	1mL	Green cover
Ultrapure water	1mL	Blue cover
Salmonella universal qPCR primer probe dry powder	50	Brown cover
Salmonella universal qPCR positive control (1 × 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°C.

Description

Salmonella spp. is a Gram negative bacterium. Salmonella infection is a zoonotic infectious diseases, which is mainly caused by eating contaminated food and is an important source of food poisoning in many countries. Typical symptoms include fever, nausea, vomiting, diarrhea, and abdominal cramps, which usually improve within 72 hours after fever. Infants, elderly people, and immunocompromised patients may experience severe and life-threatening bacteremia due to Salmonella entering the bloodstream, and a few may also develop meningitis or osteomyelitis. Therefore, rapid detection of Salmonella is of great significance for universal use.

Application

This product is a universal kit developed based on qPCR technology for the detection of Salmonella. It 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 the highly conserved region of Salmonella universal 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 fluorescent quantitative PCR 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 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

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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 dilution solution using a core gun tip, preferably using a core gun tip. The same applies below.
- 1.3. Add 5µL of 1×10^7 copy/µL positive control (provided by the reagent kit) to tube 6, shake thoroughly for 1 minute, and obtain 1×10^6 copy/µL standard curve sample. Put it on ice for later use.
- 1.4. Change the gun head and add 5µL of 1×10^6 copy/µL positive control (obtained from the previous dilution) to tube 5. Shake thoroughly for 1 minute to obtain a standard curve sample of 1×10^5 copy/µL. Put it on ice for later use.
- 1.5. Change the gun head and add 5µL of 1×10^5 copy/µL positive control (obtained from the previous dilution) to tube 4. Shake thoroughly for 1 minute to obtain a standard curve sample of 1×10^4 copy/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 the same as the starting sample volume required by the nucleic acid preparation kit, and use it 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. 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
Salmonella universal qPCR primer probe mixture	3µL	3µL	3µL
N+2 DNA samples to be tested	7µL	-	-
Ultrapure water	-	7µL	-
Step 6: Standard Curve Sample Dilution Solution (1-6)	-	-	7 µ L each (sample 2 to tube 2, sample 3 to tube 3...)

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

Process	Temperature	Time
Pre denaturation	95°C	2 min
PCR reaction	95°C	15sec
(45 cycles)	60°C	15 sec (Collect fluorescence signals from FAM channels)

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 DNA concentration of

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

- 4.2. If this kit is used for qualitative testing and only determines positive or negative, the negative control must have no Ct or Ct 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, otherwise the experiment is invalid. If the experiment is effective, analyze the sample to be tested. If there is no Ct or Ct is greater than or equal to 40, it is negative. If Ct is less than 40, it is positive.