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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Salmonella Detection Kit (RT-PCR)

Catalog No: E-FS-P004
25/50T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label on the side of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

Using real-time fluorescent PCR technology, primers and probes were designed for Salmonella specific genes. During PCR amplification, the probe bound to the template is decomposed by Taq enzyme to generate a fluorescence signal. The fluorescence quantitative PCR instrument draws a real-time amplification curve based on the detected fluorescence signal, thereby achieving qualitative detection of Salmonella at the nucleic acid level.

Kit components

The kit should be stored at -20℃ protected from light until the expiration date printed on the pouch. Repeated freezing and thawing (≥10 cycles) should be avoided.

Item	Specifications	Main Ingredients	
PCR reaction buffer	0.5/1 mL	Buffer, dNTPs, Primers, Probes, Taq DNA polymerase.	E-FS-P004
Positive Control	0.1 mL	Synthetic plasmid containing target genes.	E-FS-P004
Negative Control	0.1 mL	DNase-freeddH2O.	E-FS-P004
Manual	1 copy	/	E-FS-P004

Note: Do not mix the components from different batches of kits.

Applicable Instrument

This kit is applicable to ABI fluorescence quantitative PCR instrument series, Bio-Rad fluorescence quantitative PCR instrument series, Hongshi SLAN fluorescence quantitative PCR instrument series, etc

Materials needed but not provided

- Biosafety cabinet
- Personal protective equipment (PPE)
- General laboratory equipment (e.g.tube racks)
- Real time PCR system
- Nucleic acid extraction kit and instrument
- Pulse centrifuge
- Vortex mixer
- Real time PCR reaction tubes (0.2 mL)
- Ice-container
- Transfer pipettes (0.5 μL-1000 μL)
- Sterile tips for transfer pipettes
- Sterile tubes
- Biohazard waste container
- Refrigerator and freezer

Note

1. This product is for scientific research use only.
2. Please read the instructions for use carefully before use, and strictly follow the instructions.
3. Do not interchange components in different batches of kits.
4. Do not use expired products or products with a broken aluminum foil.
5. The specimens must be treated as potential sources of infection, and must be performed using proper PPE against biological risk according to published guidelines and local regulations.
6. Avoid the liquid in contact with eyes and skin. If it splashes onto the skin or eyes, please wash immediately with plenty of water.
7. Cap the reagents immediately after use.
8. All kit components shall be completely thawed before use.
9. The master mix must be well mixed and placed in the ice-container.
10. Use separate pipette tip for each specimen to avoid cross-contamination of specimens which could cause erroneous results.
11. Please pay attention to the note of the biosafety cabinet during the operation procedures.
12. The test accuracy is affected by the specimen collection, storage and transport process.
13. Operate in the biosafety cabinet with clean disinfection and ultraviolet sterilization to prevent the outflow of aerosol and avoid harmful substances entering the respiratory tract.
14. Perform the test in partitions (Reagent storage and preparation area, Specimen preparation area and Amplification area) and prohibit cross-movement of personnel or equipment between areas.
15. Follow the standard biosafety guidelines for handling and disposal of potentially infective material.
16. If you have any questions or suggestions during use, please do not hesitate to contact the manufacturer.
17. Each reagent is optimized for use in the E-HD-P004. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-P004 with different lot numbers.

Storage and expiry date

Storage: Store at -20℃. Must be frozen.
Expiry date: expiration date is on the packing box.

Assay procedure

Note: All work areas should be in separate (Reagent storage and preparation area, Specimen preparation area and Amplification area).
All necessary safety precautions should be taken according to the laboratory guidelines. Precautions must also be taken to prevent cross-contamination of specimens.

1. Reagent Storage and Preparation Area

1.1 Thaw the kit components at 20℃ to 25℃.

1.2 Thaw the PCR reaction buffer at room temperature, fully thaw, shake and mix well, centrifuge at low speed for a few seconds, and then divide the solution into PCR reaction tubes (20μL/tube) according to the number of samples to be amplified (sufficient for the number of samples and controls). Cap the PCR reaction tubes with care, ensure that the caps are placed correctly, and transfer to the specimen preparation area, store in a refrigerator at 4℃ away from light.

Note: It is important to thoroughly vortex the reagents. It is recommended that always taking account a surplus of 10% to compensate for pipetting inaccuracies.

2. Specimens Preparation Area

2.1 DNA extraction

DNA is extracted according to the instructions for use of the extraction kit.

2.2 The extracted DNA should be used for detection in time, otherwise it should be stored at -20℃. When using again, the extracted DNA should be fully thawed and centrifuged at 12,000 rpm for 5 minutes, and the supernatant should be used for PCR reaction.

2.3 If the extracted DNA is not used immediately, it can be stored at 2-8 ℃ for no more than 24 hours.

3. Addition of samples

3.1 Pipette 5 μL of each purified DNA sample, negative control and positive control into the corresponding PCR reaction tube containing the PCR reaction buffer.

Note: Change the pipette tip with every step.

3.2 Cap the PCR reaction tubes with care; ensure that the caps are placed correctly.

3.3 Vortex the PCR reaction tubes briefly and centrifuge to collect the solutions at the bottom.

4. Amplification Area

4.1 Put the reaction tubes on a PCR instrument, set up and run the following cycling protocol.

Step		Cycles	T (℃)	Time(s)
Step 1	Initial denaturation	1	95℃	5
Step 2	PCR amplification	45	95℃	10
Step 3			60℃	30
Step 4			72℃	30

Note: Reaction volume was set at 25μL.

a) Please set the internal reference parameter of fluorescence of the instrument to "None". For example: for ABI 7500, please set "Passive Reference" to "None".

b) Start the PCR cycler according to its user manual.

Result analysis

ABI 7500: Set Baseline to 3-15 (Baseline Cycler can be changed within a certain range according to the actual situation), and the fluorescence threshold (Threshold) setting principle is that the threshold line just exceeds the maximum value of the negative control amplification curve (irregular noise line) point, and the Ct value is displayed as Undet. Use the instrument software to automatically analyze the results.

Quality control

1. Negative control: no obvious S-shaped amplification curve, and Ct value is shown as Undet.
2. Positive control: both channels have typical S-shaped positive amplification curves, and the Ct value is less than or equal to 30.

Note: The above two conditions must be satisfied at the same time, otherwise, this test is invalid.

Reference value

1. Ct value is displayed as undet, negative result.
2. Ct value ≤ 35 positive result.

Interpretation of test results

On the premise that the experiment is valid, the test results are judged according to Table below.

Table.1 Ct value of each fluorescence channel and judgment of negative and positive results

Fluorescence channel (target gene)	**Ct value**	**Interpretation**
FAM	Ct ≥ 40（or undet）	Negative
	Ct ≤ 35，With obvious S-type amplification curve	Positive
	35< Ct < 40，With obvious S-type amplification curve	To detect the gray area, the measurement should be repeated once. If the Ct value of the retest result ≥40, it is judged to be negative.

Limitations

1. As with any molecular test, mutations within the target regions of this test kit could affect primer and/or probe binding resulting in a failure to detect the presence of a virus.
2. False negative results may occur if inadequate numbers of organisms are present in the specimen.
3. The test result dose not rule out the presence of other co-infection pathogens.