Creation date: August 2024

Test for the real-time PCR detection of *Salmonella* spp.

FS Finder SL

-*Salmonella* spp. Gene Detection Kit for Food Safety-

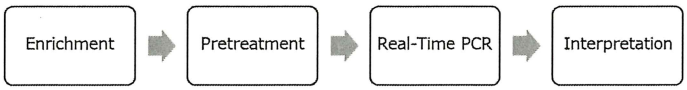
User Guide

Features & Measurement Principle

- One-step real-time PCR (probe detection method) can be used to specifically detect *Salmonella* species in enrichment culture for food and environmental specimens.
- Uses Ampdirect® technology*1 and requires no special pretreatment reagents. Simple and rapid screening for *Salmonella* species can be performed at low cost.

*1: Ampdirect (PCR buffer) suppresses the effects of PCR inhibitors contained in the specimen, allowing PCR to be performed without DNA purification.

- Includes an internal control (I.C.) as a countermeasure against false negatives. It is possible to confirm whether the PCR reaction has occurred normally for each tube.
- Employs UNG (Uracyl-N-Glycosidase) as a countermeasure against false positives due to carryover contamination.



Reagent Kit Components and Storage

Reagent Name	Feature	Quantity	Liquid volume
SL Master Mix	Brown light-shielding tube	2	950 µL
SL Enzyme Mix	Transparent tube with red cap	1	15 µL
SL Positive Control	Transparent tube with yellow cap	1	55 µL

Number of uses: 100 times
Expiration date: Shown on the package label
Storage temperature: -20°C

Additional Items Required (not included in the kit)

- Equipment and consumables

1) Sterilized Homogenize Bag with filter (Code 01540)
2) Homogenizer
3) Stand for Homogenize Bag
4) Incubator (37±1°C or 41.5±1°C)
5) Real-time PCR system: ROX and FAM fluorescent filter compatible
6) Heat block: capable of heat treatment at 95°C
7) High speed centrifuge: capable of centrifuging at 10,000 x g
8) Micropipettes and filter-fitted tips
9) Small centrifuge (for spin-down)
10) Vortex mixer
11) Refrigerants such as crushed ice or commercial refrigerants
12) Aluminum block for tube cooling
13) Reaction tubes for real-time PCR
14) Pretreatment tubes (1.5 mL to 2 mL)
15) Tubes for reaction solution preparation (0.5 mL to 2 mL)
16) Disposable gloves

- Reagents

1) Enrichment medium: Buffered Peptone Water (BPW) (Code 05121)
2) Distilled water for pretreatment (only required for specimen preparation by Method III)

Operating Procedure

1. Operating Precautions

1) The reagent kit should be stored frozen (-20°C).
2) All reaction solutions should be prepared under ice-cold conditions (on an aluminum block cooled with crushed ice or other refrigerant).
3) SL Master Mix and SL Positive Control should be thawed at room temperature, thoroughly mixed and spun down with a vortex mixer, and kept under ice-cold conditions until use.
4) SL Enzyme Mix does not freeze when refrigerated and does not need to be thawed at room temperature. Mix and spin down thoroughly with a vortex mixer and keep under ice-cold conditions until use.
5) Pathogen testing should be performed in a properly equipped laboratory under the supervision of a trained laboratory technician.
6) When handling reagents and specimens, countermeasures against biohazards should be taken, such as wearing appropriate protective clothing and safety glasses.
7) Always wear gloves to prevent exposure to biohazards and to avoid contamination.
8) Laboratory workbenches, pipettes, and other equipment should be cleaned regularly with a household bleach solution diluted to 1-5% (v/v) with water or DNA removal solution.

2. Specimen Preparation and Enrichment

- Solid foodstuffs

1) Weigh 25 g of specimen into a sterile homogenizer bag with filter.
2) Add 225 mL of separately prepared Salmonella enrichment broth (Buffered Peptone Water) and homogenize for about 1 minute.
3) Incubate the mixed solution at 37±1°C or 41.5±1°C, for 6 to 30 h*2.

- Water or liquid foodstuffs

1) Add 9 times the volume of the Salmonella enrichment broth to the specimen volume.
2) Filter the specimen through a membrane filter, then directly add the filter to the Salmonella enrichment broth.
3) Incubate the mixed solution at 37±1°C or 41.5±1°C, for 6 to 30 h*2.

- Wiped samples

1) Add 9 times the volume of the Salmonella enrichment broth to the total volume of liquid that has been wiped off the food or environmental material with a cotton swab, etc.
2) Incubate the mixed solution at 37±1°C or 41.5±1°C, for 6 to 30 h*2.

*2: The detection sensitivity depends on the type of food, medium used, incubation temperature, and incubation time. The user should determine the protocol after considering the enrichment conditions at their laboratory.

3. Pretreatment of enriched specimen (3 methods)

Pretreat the enriched specimen prepared in [1. Specimen Preparation and Enrichment] by one of the following methods, I-III. As well as the enriched specimen, it is recommended to perform the same pretreatment on the enrichment broth and use this as a negative control sample.

■ Method I

1) The enriched specimen is used directly for real-time PCR. No pretreatment is performed.

■ Method II

1) Place 1 mL of the enriched specimen in a pretreatment tube.
2) Incubate the tube in a heat block at 95°C for 5 min.
3) The supernatant (avoid aggregates) is used for real-time PCR*3.

■ Method III

1) Place 1 mL of the enriched specimen in a pretreatment tube.
2) Centrifuge at 10,000 x g for 1 minute*4.
3) Discard the supernatant and add 1 mL of distilled water.
4) Resuspend the pellet in a vortex mixer.
5) Incubate the tube in a heat block at 95°C for 5 min.
6) The supernatant (avoid aggregates) is used for real-time PCR*3.

*3: Centrifuge for 1 minute (up to 5000 x g) if the supernatant is difficult to collect.
*4: Centrifugation time may not be sufficient for some samples. In such case, extend the time to a maximum of 5 minutes.

4. Preparation of reaction solution

1) Prepare the reaction solution in a tube*5.
[For one test]
: Calculate the amount used by multiplying by the number of reactions required.

SL Master Mix		18	µL
SL Enzyme Mix*5		0.1	µL
	Total	18.1	µL

*5: Handle the SL Enzyme Mix carefully because the amount used is very small.

After mixing each reagent, mix thoroughly with a vortex mixer for 5 seconds.
It is recommended to increase the reaction numbers by 10% to account for pipetting errors.

2) Add 18 µL of the reaction solution prepared in the preceding step 1) to the PCR reaction tube.
3) Add 2 µL of the pretreated sample prepared in 2 to the PCR reaction tube.
When using SL Positive Control, add 2 µL in place of the pretreated sample.
4) Mix thoroughly with a vortex mixer for 5 seconds, spin down, and immediately start the reaction on the real-time PCR system.

5. Real-time PCR reaction

1) The setting conditions for real-time PCR are as follows.

[Setting Condition]

When using QuantGene 9600 (Hangzhou Bioer Technology)

Temperature	Time
95°C	3 minutes
	↓
[94°C	1 sec ⇒ 60°C, 30 sec*6] × 45 cycles (photometry)

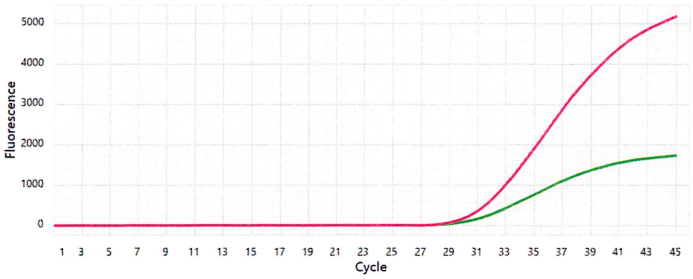
*6: Conduct photometry with ROX and FAM fluorescence filters at the 60°C step. Optimization of real-time PCR amplification conditions and optical settings (gain, etc.) may be necessary depending on the device used. Verify the settings before use. Use the device correctly according to its instruction manual.
2) Confirm the amplification curve from the ROX and FAM fluorescence filters and interpret as follows.

[Interpretation]

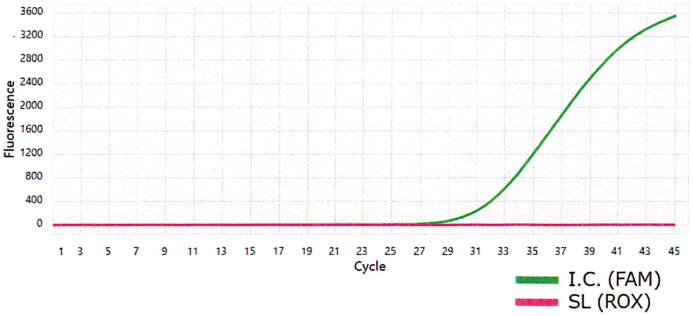
■ Controls

Verify the positive (SL Positive Control) and negative (distilled water or BPW) controls before interpreting sample results. For the experiment to be valid, the controls must have the following results, as shown in the figures below.

Positive control



Negative control



*These figures were acquired using a QuantGene 9600 (Hangzhou Bioer Technology).

■ Sample

Interpret the sample results, as summarized in the table below.

Interpretation	*Salmonella* species (ROX)	I.C. (FAM)
Positive*7	○	× or ○*8
Negative	×	○
Not determinable*9	×	×

*7: When this kit returns a positive result, perform a conventional confirmation test by culturing separately from the enrichment medium.
*8: I.C. amplification may be suppressed by high concentrations of *Salmonella* species.
*9: If neither of the amplification curves can be confirmed, reanalysis is required.

Precautions

1. Reagents

1) This product is for research use only. It is not intended for therapeutic diagnostic purposes or procedural use.
2) Use the product correctly in accordance with these instructions.
3) This reagent kit does not guarantee the complete detection of *Salmonella* species. Detection may not be possible due to the number of bacteria or contaminants in the sample or depending on the mutant strain.

2. Disposal

1) To prevent contamination by amplified products, discard the reaction tube without opening the lid after completing PCR. Do not autoclave when disposing of the tubes; DNA is not degraded by autoclaving. Aerosols may be generated and cause contamination.
2) Dispose of waste properly in accordance with national and local laws and regulations.

3. Other

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Warranty

1. Warranty

Performance is guaranteed as described in the instruction manual. The expiration date is indicated on the label on the package. The product should be used by the expiration date.

2. Limitation of Liability

In no event shall we be liable for loss of profits, or indirect or consequential damages incurred by the customer. We shall also not be liable for any damages based on compensation made by a third party to the customer. The presence or absence of a response to this product may vary depending on the customer's operating methods and measurement devices. We shall not be liable for any damages incurred even in the event of misjudgment. In any case, our liability for damages shall be limited to the amount equivalent to the price of this product.

3. Warranty Exclusions

The following cases are excluded from the warranty, even if the quality of the product is found to be defective before the expiration date: (1) if the product is used incorrectly, (2) if the product is stored incorrectly, or (3) if the defect is caused by a reason not attributable to the product.

Package

FS Finder SL 100 tests ..... Code 69411

Further Information

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