Kaneka

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Instruction Manual

Kaneka Nucleic Acid Chromatography Food Poisoning Bacteria Detection Kit (Salmonella sp./O4 group / O7 group / O9 group) version 2

Caution

- ■This product is for research purposes only. Please do not use for medical care for humans or animals, clinical diagnosis, etc.
- ■Retain the various reagents of this product in strict accordance with the storage protocols described in this instruction manual. Be careful of contamination when storing.
- ■The specifications of this product are subject to change without notice.
- ■Please use the equipment, devices, reagents (including culture medium) used for the preparation and reaction of PCR solution according to this instruction manual and the usage protocols specified by each manufacturer/distributor.
- ■Please be responsible for the diagnosis and use of the results obtained by this product. We will not be liable for any damages or losses incurred by you because of implementation.
- ■The customer should verify the operation procedure not described in this instruction manual and the validity of the detection result for each sample.

Features/Contents

This product is a kit for detecting Salmonella and O4, O7, and O9 groups of Salmonella, which are known to cause food poisoning, quickly and easily. This product contains amplification enzyme mix, primer mix, test strip (5 lines), and chase buffer. Please prepare media and DNA extraction reagent (if necessary) separately.

Component	Contents (100 tests)
Amplification enzyme mix	1 mL x 1
Primer mix	0.8 mL x 1
Test strip (5 lines)	50 strips x 2 bottles
Chase buffer	16 mL x 1
Instruction Manual	1 manual

Storage Conditions/Expiration

■ How to Store

Please avoid direct sunlight

- ･Amplification enzyme mix: -20℃ storage
- ･Primer mix: -20℃ storage
- ･Test strip (5 lines): Store at room temperature
- ･Chase buffer: Store at room temperature

【Precautions】

- If the test strip (5 lines) contains moisture for a long time, the detection performance may deteriorate, so put it in the container (including the desiccant) after opening and store it with the lid tightly closed to prevent absorbing moisture.
- For amplification enzyme mix and primer mix, detection performance may deteriorate if freeze-thaw is repeated, so all is not used at one time, we recommend to store by dispensing and minimizing the freeze-thaw cycle as much as possible.

■ Expiration Date

Stated on the packaging of this product.

How to Use

■ **Equipment and device required in addition to this product**

1. Micropipette
2. Micropipette with filtered tip
3. Thermal cycler (Recommended device: LifeECO Thermal Cycler (Bioer Technology))
4. 1.5 mL tube
5. 0.2 mL tube（For PCR）
6. Small incubator (for 1.5 mL, capable of heating above 95℃)
7. Low speed centrifuge

■ Culture

Please perform the following culture method.

<table>
<tr><th>Sample Types</th><th>Culture method (1st phase)</th><th>Culture method (2nd phase)</th></tr>
<tr><td>Liquid egg</td><td>Add 25 g of liquid egg to 225 mL of buffered peptone water (BPW) and incubate at 37℃ for 19 hours or longer.</td><td rowspan="2">Add 10 μL of BPW (supernatant) after culturing to 500 μL of Brain Heart Infusion (BHI) medium and incubate at 37°C for 3-4 hours.</td></tr>
<tr><td>Feed</td><td>Add 25 g of feed (feed ingredients, mixed feed) to 250 mL of buffered peptone water (BPW) and incubate at 37°C for 19 hours or longer.</td></tr>
<tr><td>Raw meat</td><td>Add 25 g of raw meat to 225 mL of buffered peptone water (BPW) and incubate at 37°C for 22 hours or longer.</td><td>—</td></tr>
<tr><td>Feces/meconium</td><td>Add 25 g of feces and meconium to 225 mL of buffered peptone water (BPW) and incubate at 37°C for 22 hours or longer.</td><td>—</td></tr>
<tr><td>Intestinal swab (broiler)</td><td>Put 5 swabs swabbed in the intestine (rectal) in 10 ml Hana Tetrathione (HTT) medium and incubate at 42°C for 22 hours or longer.</td><td>—</td></tr>
</table>

■ DNA extraction from culture

Please extract DNA from the culture broth according to the following method.

<table>
<tr><th>Sample Types</th><th>DNA extraction method</th></tr>
<tr><td>Liquid egg</td><td rowspan="2">・ Set the small incubator to 98°C and heat it before the inspection.
・ Dispense 100μL of Solution A of Kaneka Simple DNA Extraction Kit ver.2 in a 1.5 mL tube (for each sample). Add 50μL of culture solution (supernatant) to it.
・ Heat the 1.5mL tube containing the sample at 98°C for 8 minutes.
・ After heating, return the 1.5mL tube to room temperature, add 14μL of Solution B of Kaneka Simple DNA Extraction Kit ver.2, and mix.
・ Use the supernatant as the DNA extract.</td></tr>
<tr><td>Feed</td></tr>
<tr><td>Raw meat</td><td rowspan="3">・ Preheat the small incubator to 98°C before testing.
・ Dispense 900μL of 1 x TE buffer into a 1.5 mL tube (for each sample). Add 100μL of culture solution (supernatant).
・ Heat the 1.5mL tube containing the sample at 98°C for 10 minutes.
・ Use the supernatant as the DNA extract.</td></tr>
<tr><td>Feces/meconium</td></tr>
<tr><td>Intestinal swab (broiler)</td></tr>
</table>

- **Preparation of PCR solution**
  - Thaw the frozen primer mix* and amplification enzyme mix* in advance.
  - Dispense and mix 8 μL of primer mix and 10 μL of amplification enzyme mix into a 0.2 mL tube to prepare a master mix.

    *Before dispensing, mix the primer mix and amplification enzyme mix thoroughly by pipetting. If the amplified enzyme mix shows a white precipitate after thawing, completely dissolve it before use.

Primer mix	8 μL
Amplification enzyme mix	10 μL

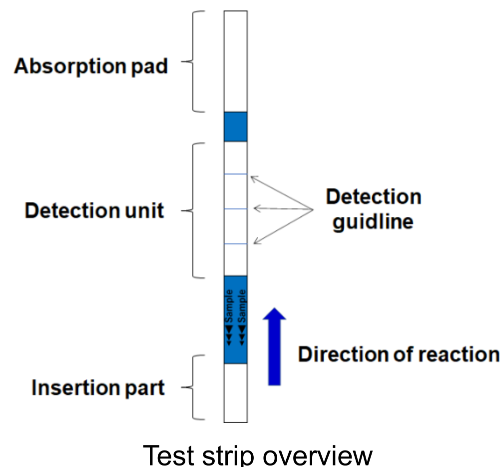
  - Add 2 μL of DNA extract to the 0.2 mL tube in which the master mix was prepared.
  - After preparation, place on ice, or something cold, to prevent enzyme inactivation and non-specific amplification reaction.

- PCR
  - Start the thermal cycler, set the 0.2 mL tube, and then perform the amplification reaction with the following program.

Steps	Temp./Time	# of Cycles
Step 1	25°C / 10 min	1
Step 2	94 °C / 2 min	1
Step 3	94 °C / 5 sec →	35
	61 °C / 35 sec	
	72 °C / 20 sec	
Step 4	4 °C / （※）	1

【Precautions】
- Immediately after preparing the PCR solution, perform the reaction in the thermal cycler.
- When preparing the reaction solution, use a micropipette with sterilized filtered tip.
- (*) After completion of the reaction, remove the 0.2 mL tube on the day and perform detection with a test strip.

- Detection by test strip
  - After the PCR, remove the 0.2 mL tube from the thermal cycler, open the lid of the 0.2 mL tube, and add 160μL of chase buffer.
  - Insert the test strip into a 0.2 mL tube, allow the part with the sample of the test strip to soak in the reaction solution, and leave it as it is.
  - After 10 minutes, visually analyze the control line and each detection line.

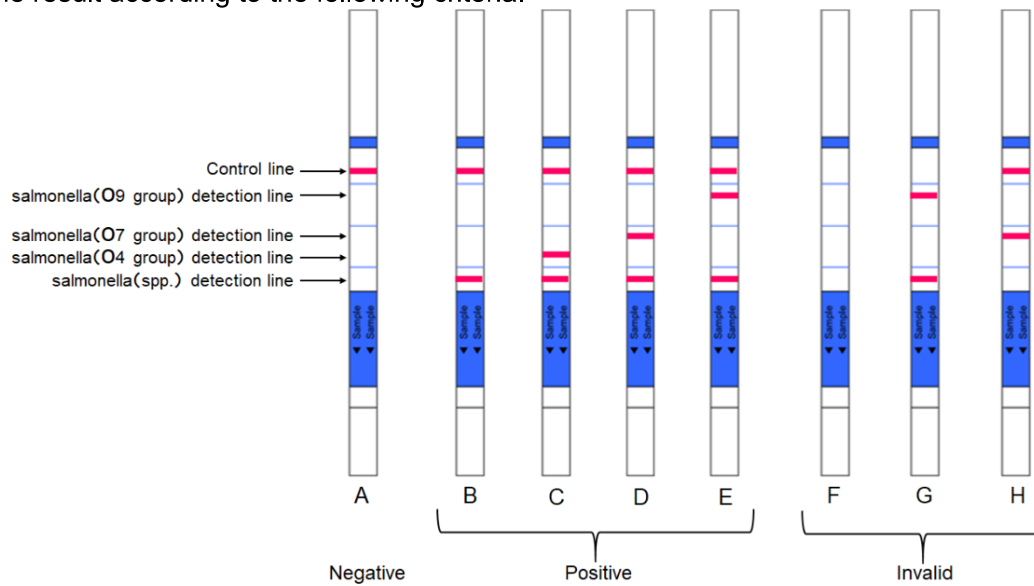


Test strip overview

【Precautions】
- 【Precautions】
- Determine by line coloring 10 minutes after insertion.
- Wear gloves when handling the test strip and always hold the top part of absorbent pad. Be careful not to touch the test strip insertion part (part with sample) and detection unit with your hands.
- When inserting the test strip into the tube, be sure to insert it firmly until the part with the sample hits the bottom of the 0.2 mL tube.
- When inserting the test strip, be careful of contamination due to splashing of the reaction solution.

■ Determining the Result

Judge the result according to the following criteria.



Example of results

Pattern A : Salmonella not detected
Pattern B : Detects Salmonella that do not belong to O4, O7, and O9 groups
Pattern C : Detects Salmonella belonging to O4 group
Pattern D : Salmonella belonging to O7 group is detected
Pattern E : Detects Salmonella belonging to O9 group
Patterns F to H (example): Unable to detect (retest required)

【Precautions】
- The control line is always colored when the amplification reaction is proceeding normally. If the control line does not stain, the amplification reaction may not have proceeded normally, so please perform a retest.
- Each detection line will only be colored if it is positive. However, if the O4, O7, and O9 group detection lines are colored, the Salmonella detection line is also colored. If the O4, O7, and O9 detection lines are colored but the Salmonella detection line is not colored, it has failed to detect so please perform a retest. (Use the same DNA extract when retesting).

Notes on using this kit

<Contamination measures>

■ To prevent misjudgment due to contamination, replace the used micropipette tip every time. Also, use a micropipette tip with a filter.

■ We recommend that each step of DNA extraction, PCR solution preparation, and test strip detection be performed in separate laboratories. If it is difficult to carry out in separate laboratories, separate workbenches, or work areas within the same laboratory.

- Before opening the tubes for reagents, extracted DNA, etc., spin them down with a low speed centrifuge. (To prevent the contents from scattering and reagents attached to the lid from sticking to fingers)
- Regularly clean micropipettes and other equipment and devices used with 0.1% sodium hypochlorite and commercially available DNA removers.
- If contamination occurs, decontaminate with 0.55% sodium hypochlorite (or a commercially available DNA remover) or UV irradiation.

＜Disposal precautions＞

- When discarding the used test strip and amplification solution, be careful not to touch the detection part and put them in a plastic bag, etc. before discarding.
- When discarding the sample after DNA extraction, please take into consideration the sanitary and environmental aspects in accordance with the local regulations on waste and the regulations of the facility.

＜Performance＞

- Proper detection results can be obtained when detection is performed on the genomic DNA (5 pg/test or more) extracted from Salmonella strains according to the method described in this instruction manual.
- We recommend using this product with Life ECO Thermal Cycler (Bioer Technology). The detection sensitivity of this product may differ depending on the thermal cycler used.
- Detection sensitivity may differ depending on the DNA extraction method.
- If the amount of DNA after DNA extraction is extremely large or if the amount of culture medium brought in is large, the PCR amplification reaction may be blocked, and each detection line and control line may not be detected. In that case, it may be improved by diluting the DNA extract for PCR.

Assurance

- Our responsibility is limited to the replacement of the product with a defective product, and we will not be liable for any other damage, whether direct or indirect. Please be aware of this before using this product.

Contact Information

Kaneka Corporation Medical Solutions Vehicle

2-3-18, NAKANOSHIMA, KITA-KU, OSAKA, 530-8288, JAPAN

URL https://www.kaneka-labtest.com