

Reagent for food inspection

NH IMMUNOCHROMATO SALMONELLA <<Instruction Manual>>

* Please read this manual carefully before using the kit.

[Development history]

Salmonella food poisoning is one of the most important food poisoning in the world. In recent years, *Salmonella* has been one of the leading causes of food poisoning in Japan: a total of 144 food poisonings affected 3,700 people, resulting in one death in 2005.

Salmonella has more than 2,500 serotypes¹⁾, about 1,500 of which are pathogenic to humans and animals. Among them, *S.Enteritidis* has been rapidly emerging as a cause of food poisoning through chicken eggs and their products: food poisoning by *S.Enteritidis* accounts for 50% to 62% of all *salmonella* food poisonings²⁾.

This kit is designed to detect *S.Enteritidis*, the most problematic bacterium among *salmonella* in food and provide a test result in a short time with only a simple method due to its immuno-chromatographic principle.

[Characteristics]

- 1) This product does not need skill because it can be handled only with a one-step procedure.
- 2) It rapidly provides a test result.
- 3) It needs no specific detector.

[Contents of kit]

1) Components

- A: Test plate: 5 tests × 4
B: Instruction manual

2) Components and volume (per test)

- | | |
|---|----------|
| (1) Anti- <i>Salmonella</i> polyclonal antibody (rabbit) | 0.25 µg |
| (2) Gold colloid labeled anti- <i>Salmonella</i> polyclonal antibody (rabbit) | 0.075 µg |
| (3) Anti-rabbit immunoglobulin polyclonal antibody (goat) | 0.25 µg |

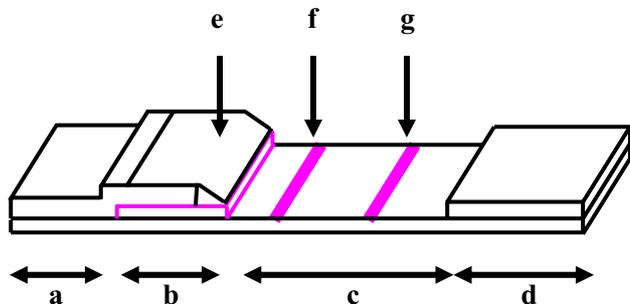
[Objective]

- 1) Detection of *Salmonella* (*S.Enteritidis*) in food products

Note) This kit is intended to specifically detect *S.Enteritidis* and therefore cannot detect other serotypes of *Salmonella*.

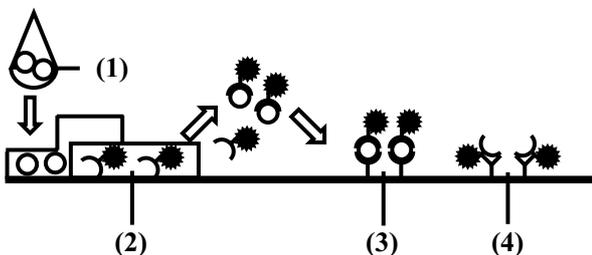
[Test plate part name and detection principle]

1) Part names



- a. Sample dropping part (Take care not to touch it with your hands)
- b. Reagent containing part
- c. Spread area (Take care not to touch it with your hands or damage it)
- d. Absorption pad
- e. Measurement item entering area
- f. Test line appearance position (about 30 mm from the sample dropping part)
- g. Control line appearance position (about 38 mm from the sample dropping part)

2) Detection mechanism



When a sample solution is dropped at the sample dropping part of the test plate, the gold colloid labeled anti-*Salmonella* antibody (2) is dissolved to form complexes with *Salmonella* (1) in the sample solution. The complexes migrate across the spread area by capillarity and are then trapped by the anti-*Salmonella* antibody (3) fixed at the test line appearance position to develop a reddish purple line by the gold colloid. Thus, whether the sample solution contains *Salmonella* or not can be determined by visually inspecting the test line appearance position.

Irrespective of the presence/absence of *Salmonella* in the sample solution, the excess gold colloid labeled antibody further migrates across the spread area and is then trapped by the anti-rabbit immunoglobulin goat antibody (4) fixed at the control line appearance position to develop a reddish purple line. It can be confirmed that the sample solution has migrated across the spread area by the development of the line.

[Preparation of sample solution]

*: The preparation of the sample solution is based on the Guidelines on Food Hygiene Testing³⁾.

1) Necessary apparatus and equipment

Stomacher bag (that with a filter is recommended), stomacher, incubator ($35.0 \pm 1.0^\circ\text{C}$ and $43.0 \pm 1.0^\circ\text{C}$), autoclave, enrichment medium, etc.

2) Sample preparation

- (1) When the food is solid, collect small samples from as many sites as possible to make up a solid sample of 25 g. When the food is liquid, stir it well before collecting a sample of 25 mL.

3) Enrichment culture

- (1) Add the sample to the pre-enrichment medium (buffered peptone water, EEM bouillon, and lactose bouillon) at a volume that is nine times as much as the sample. Treat it with a stomacher for an appropriate time depending on the sample and culture it at $35.0 \pm 1.0^\circ\text{C}$ for 18 ± 2 hours.
- (2) Add 1 ml of the pre-enrichment culture solution to 15 ml of the selected enrichment medium (Rappaport-Vassiliadis [RV] culture medium, selenite brilliant green [SBG] culture medium, and Hajna tetrathionate [TT] culture medium) and culture it at $43.0 \pm 1.0^\circ\text{C}$ for 18 hours.

Note 1: When a liquid egg is used as a sample, use a buffered peptone water containing L-cysteine (0.2 g/L) or $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (64 mg/L) as a pre-enrichment medium and culture it at $36.0 \pm 1.0^\circ\text{C}$ for 22 ± 2 hours.

Note 2: When a liquid egg is used as a sample, inoculate 0.5 mL of the pre-enrichment culture solution to 10 mL each of the RV and TT culture media and culture them at $42.0 \pm 0.5^\circ\text{C}$ for 22 ± 2 hours.

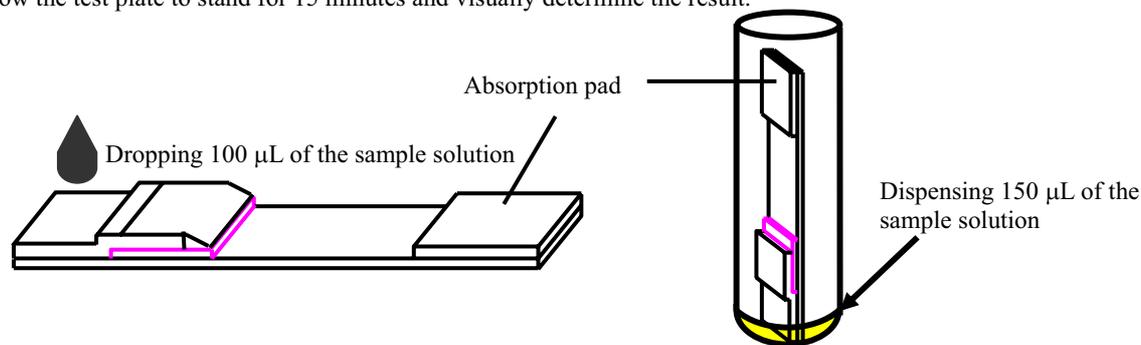
Note 3: Keep the remaining culture solution safely without sterilizing it until the end of the test because it may be used for confirming the test result of the kit.

Note 4: Use the culture solution without sterilization with full attention to prevent infection because sterilizing the culture solution may reduce detection sensitivity.

[Test procedure]

1) Test procedure of NH IMMUNOCHROMATO Salmonella

- (1) Allow test plates to room temperature without removing them from the aluminum pouch and remove a necessary number of the test plates from the pouch immediately before use.
- (2) Write the sample name or specimen number on the absorption pad of each test plate using a felt pen.
- (3) Place the test plate on a horizontal plane and drop 100 μL of the sample solution onto the sample dropping part (see the following left figure). Alternatively, dispense 150 μL of the sample solution into a test tube and place the test plate into the test tube so that the sample dropping part will be immersed in the sample solution (see the following right figure).
- (4) Allow the test plate to stand for 15 minutes and visually determine the result.



Note 1: Since moisture may adversely affect the performance of the test plate, remove it from the aluminum pouch after it is allowed to room temperature.

Note 2: Return any unused test plate into the plastic pouch with a desiccating agent and put the pouch into the aluminum pouch. Keep the aluminum pouch in a refrigerator.

Note 3: Take care not to touch or damage the sample dropping part and spread area of the test plate. Hold the test plate by the absorption pad.

Note 4: Use only a sterilized pipette or tip to drop or dispense the sample solution. Do not use one pipette or tip for more than one sample solution.

Note 5: Take care not to make the sample solution overflow from the test plate when dropping 100 μL of the sample solution. Take appropriate measures for preventing the overflow, such as dividing the solution into two portions and dropping them separately, as required.

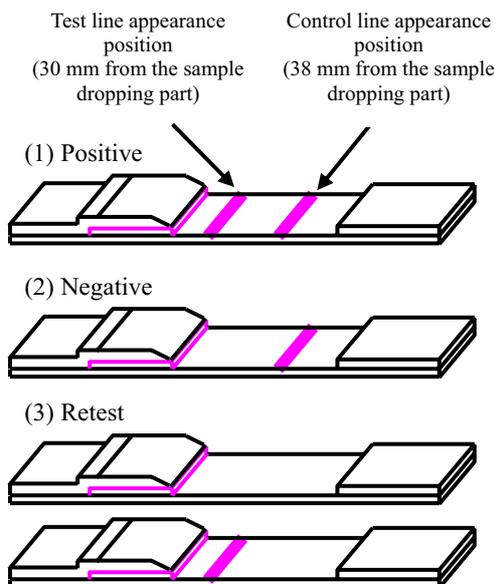
Note 6: To prevent infection, spread a plastic wrap or equivalent and place a test plate on it when you choose to perform the test by dropping the sample solution onto the test plate.



3) Determination of result

- (1) Determine the sample as positive when a reddish purple line is observed at both the test line appearance and control line appearance positions 15 minutes after the test is started. When no or only a light reddish purple line is observed at the test line appearance position at that time, check the position again 60 minutes after the test is started.
- (2) Determine the sample as negative when a reddish purple line is not observed at the test line appearance position, but is observed only at the control line appearance position.
- (3) When no reddish purple line is observed at the control line appearance position, repeat the test, irrespective of the presence/absence of a reddish purple line at the test line appearance position because the spread of the sample solution may have been abnormal.

Note 1: For the sample determined as positive by this kit, confirm the result with other test methods, such as the official inspection method. The sample cultured in the enrichment medium for this kit can be used for confirmation tests, such as the official inspection method.



[Performance]

1) Sensitivity test

When a sample containing *S. Enteritidis* ATCC 13076 is tested according to the "Preparation of sample solution" and "Test procedure" of the present instruction manual, a positive test result is obtained when the bacterial concentration of the sample is 1×10^6 CFU/mL or higher.

2) Reproducibility test

When *S. Enteritidis*-positive and -negative sample solutions are simultaneously tested three times each, the positive solution is determined as positive and the negative solution as negative for all the repetitions.

3) Minimum detection sensitivity

It was confirmed from a test using two standard and three isolated strains of *S. Enteritidis* that the minimum detection sensitivity of the kit ranged from 1×10^5 to 1×10^7 CFU/mL.

Note 1: The minimum detection sensitivity of the kit may vary depending on the strains of *S. Enteritidis*, type of the enrichment culture medium, and sample components.

4) Cross-reactivity

- (1) No cross-reactivity has been detected with the following strains.

	Control number	Result
<i>Escherichia coli</i>	ATCC 1175	-
<i>Citrobacter freundii</i>	ATCC 8090, 43864	-
<i>Enterobacter aerogenes</i>	ATCC 13048	-
<i>Enterobacter cloacae</i>	ATCC 13047, 49141	-
<i>Enterobacter sakazakii</i>	ATCC 51329	-
<i>Klebsiella oxytoca</i>	ATCC 8724	-
<i>Klebsiella pneumoniae</i>	ATCC 4352	-
<i>Serratia liquefaciens</i>	ATCC 27592	-
<i>Serratia marcescens</i>	ATCC 8100	-
<i>Serratia odorifera</i>	ATCC 33077	-

- (2) Among serotypes other than *S. Enteritidis*, this kit has been confirmed to respond to *S. abaeetuba* and *S. anatum*.



[Precautions for use and handling]

1) Precautions for use

- (1) Read the instruction manual carefully before using this kit. Follow the instructions of the manual.
- (2) Do not use the kit after its expiration date indicated in the aluminum pouch label.
- (3) This kit is intended for detecting *S. Enteritidis* in food and not intended for making a clinical diagnosis.
- (4) This kit may show a false-positive result due to the effect of sample components. For the sample determined as positive by this kit, confirm the result with other test methods, such as the official inspection method.
- (5) For the equipment and reagents (including culture media) used for preparing a sample solution, follow the instructions provided by their manufacturers or distributors.
- (6) This instruction manual is intended to be a guideline for inspectors. Verify the appropriateness of the procedure of the manual and the applicability of this kit to each food.
- (7) The specifications of the kit are subject to change without prior notice.

2) Precautions for preventing risk

- (1) Take appropriate safety measures in performing the test, such as putting on protective gloves and glasses, because *Salmonella*, the target bacteria of this kit, may cause infection even at a trace level and because other microbes can cause infection.
- (2) Use this kit with appropriate equipment and facilities under the supervision of an eligible manager according to an appropriate standard microbiological test procedure.
- (3) When the sample solution accidentally enters the eye or mouth, take immediate emergency measures, such as rinsing it with tap water, and receive medical attention.
- (4) Immediately receive medical attention if you feel any physical abnormality after using this kit.

3) Precautions for disposal

- (1) Since the test plates, enrichment media, and any remaining sample or sample solution used for the test may be infectious, sterilize them with an appropriate method, such as autoclaving them at 121°C for 20 minutes or immersing them in a 0.1% sodium hypochlorite solution for at least one hour.
- (2) Dispose of this kit and any remaining sample or sample solution according to local regulations on waste disposal with full attention to the possible effect on sanitation and environment.

[Storage and expiration date]

- 1) Storage: Keep this kit refrigerated (2 to 8°C) in a dark place. Take care not to freeze it.
- 2) Expiration date: 12 months after the date of manufacture

[Package unit]

NH IMMUNOCHROMATO Salmonella for 20 tests

[References]

- 1) Popff, M.Y.: Antigenic formulas of the *Salmonella* serovars, 8th ed, WHO Collaborating Centre for Reference and Research on *Salmonella*, Institute Pasteur (2001).
- 2) National Institute of Infectious Diseases: Pathogenic microbe detection information, Vol. 27, No. 8, 2006.
- 3) Japanese Ministry of Health, Labour, and Welfare: Guidelines on Food Hygiene Testing, Volume of Microorganisms, Japan Food Hygiene Association, 180-191 (2004).

[Manufacturer]

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