NH IMMUNOCHROMATO VT 1/2 <<Instruction Manual>>

*Please read this manual before using this kit.

[Introduction]

Enterohemorrhagic *Escherichia coli* (*E. coli*), which has been reported in association with extensive food poisoning or deaths, induces symptoms by producing verotoxins (VTs). The verotoxins are broadly classified into verotoxin type 1 (VT1) and verotoxin type 2 (VT2), which are known to have very strong toxicity.

In Japan, a number of serotypes have been detected such as O157 and O26. For these serotypes, test methods have already been provided through notification from the regulatory authority. For other *E. coli* serotypes, examination of verotoxins in food is effective in preventing enterohemorrhagic food poisoning. Furthermore, a verotoxin test should be performed if enterohemorrhagic *E. coli* is detected because verotoxin production is used to confirm enterohemorrhagic *E. coli*.

This product is a kit for detecting verotoxin using immunochromatography. With this kit, simple tests can be conducted rapidly to differentiate VT1 and VT2.

[Product Features]

1) The simple one-step operation of the kit.
2) The test gives rapid results.
3) There is no need for special test equipment.

[Kit Contents]

1) Components
   A: Test strip 2-test × 10 packs
   B: Instruction manual 1 sheet
   C: Plastic pouched bag 1 bag

2) Ingredients
   (1) Reagent-containing section
      Gold colloid-labeled anti-VT1 antibody (rabbits)
      Gold colloid-labeled anti-VT2 antibody (rabbits)
   (2) Detecting section
      Anti-VT1 antibody (rabbits)
      Anti-VT2 antibody (rabbits)
      Anti-rabbit immunoglobulin antibody (goat)

[Application]

1) Detection of VT1 and VT2 in Foods
2) Examination of VT1 and VT2 production from isolated enterohemorrhagic *E. coli*.

[Illustration of Test strip and the Principle of assay]

1) Illustration of Test strip

2) Principle of assay

When a sample solution is dropped onto the sample solution drop section, gold colloid-labeled anti-VT1 antibodies and gold colloid-labeled anti-VT2 antibodies in the Reagent-containing section dissolve and form complexes with verotoxins (1). These complexes move to the detecting section by capillary attraction and are trapped by anti-VT1 or anti-VT2 antibodies (3) that is fixed in the test line appearance position. This results in the appearance of a reddish purple line of gold colloid. This reddish purple line can be detected by visual inspection and used to judge the presence or absence of VT1 and VT2 in the sample solution.

The excess gold-labeled antibodies, regardless of the presence or absence of verotoxin in the sample solution, travel further through the detecting section and are trapped by the anti-rabbit immunoglobulin antibodies (4) fixed at the control line appearance position, where they form a second reddish purple line. The presence of this line indicates that the sample solution has reached the detecting section.
[Preparation of the sample solution 1 (detection in food)]

1) Required Equipment and Instruments
Stomacher bag (preferably with a filter), stomacher, incubator, autoclave, micropipettes and tips, centrifuge, constant temperature water bath, mEC broth with novobiocin and polymyxin B solution, etc.

2) Preparation of Test Samples
(1) Weigh out 25 g of test food in stomacher bag to use as specimen.
(2) Add 225 mL of mEC broth with novobiocin to the 25g specimen in stomacher bag and homogenize with a stomacher for 1 minute.
(3) Incubate the specimen in the stomacher bag at 42°C for 18–24 hours.
(4) Remove the stomacher bag from the incubator after 18-24 hours. Gently mix the contents of the stomacher bag, taking care not to splash it.
(5) Dispense 1 mL of the culture medium into sterilized vessels using a sterilized pipette.
(6) Add polymyxin B solution to the dispensed culture medium so that the final concentration is 0.5 mg/mL.
(7) Warm the culture medium supplemented with polymyxin B solution in a constant temperature water bath set at 37 °C for 30 minutes.
(8) After 30 minutes, bring the culture medium to room temperature to use the culture medium as the sample solution.

Note 1: When performing the test, take preventive measures against infection such as wearing protective groves and glasses, because infectious samples solutions are used.

Note 2: It is recommended that incubate the suspension or the culture 37°C with the shaking every 5 to 10 minutes.

Note 3: It is recommended that centrifuge the suspension or the culture at 1,500 × g for 15 minutes after incubate.

Note 4: Because the remainder of the culture solution might be required for use in confirmatory tests following those conducted with the kit, do not sterilize it and retain it until all the tests have been completed.

[Preparation of Sample Solution 2 (detection in isolated colony)]

1) Required Equipment and Instruments
Incubator, autoclave, micropipettes and tips, centrifuge, constant temperature water bath, CAYE culture medium, CAYE broth supplement, culture medium for enterohemorrhagic E. coli, and polymyxin B solution, etc.

2) Preparation of Test Samples
2)-1 If isolation medium for enterohemorrhagic E. coli is used:
(1) Inoculate the test colony onto the isolation medium for enterohemorrhagic E. coli and incubate it at 37 °C for 18 to 24 hours.
(2) After the incubation, collect colonies on the medium surface (one third to all surface of the plate medium) and suspend them in 1 mL of 0.5 mg/mL polymyxin B solution.
(3) Incubate the suspension in a constant temperature water bath set at 37 °C for 30 minutes.
(4) After 30 minutes, bring the suspension to room temperature to use the suspension as the sample solution.

2)-2 If CAYE medium is used:
(1) Inoculate the test colony into 1 mL of CAYE medium (containing CAYE broth supplement) and incubate at 37 °C for 6 to 24 hours.
(2) After the incubation, add polymyxin B solution so that the final concentration is 0.5 mg/mL.
(3) Incubate the culture supplemented with polymyxin B solution in a constant temperature water bath set at 37 °C for 30 minutes.
(4) After 30 minutes, bring the culture to room temperature to use the culture medium as the sample solution.

Note 1: When performing the test, take preventive measures against infection such as wearing protective groves and glasses, because infectious samples solutions are used.

Note 2: It is recommended that incubate the suspension or the culture 37°C with the shaking every 5 to 10 minutes.

Note 3: It is recommended that centrifuge the suspension or the culture at 1,500 × g for 15 minutes after incubate.

[Operating Procedures for Testing]

1) NH Immunochromato VT 1/2 Test Procedures
(1) Bring the test strip contained in the aluminum pouch to room temperature and remove from the pouch immediately before use.
(2) With an oil-based marker pen, write the name of the test sample or the number of the subject under test on the absorbent pad of the test strip removed from the pouch.
(3) Place the test strip carefully on the flat stand and drop a 100 µL-portion of the sample solution onto the sample solution drop section (see the figure on the left). Otherwise, dispense a 150 µL-portion of the sample solution into a test tube and attach the test strip to the test tube so that the test sample drop section of the test strip is immersed in the sample solution (see the figure on the right).
(4) Allow the test strip to stand undisturbed for 15 minutes and then visually judge the presence or absence of VT 1/2 in the solution.
Note 1: Do not remove the test strip from the aluminum pouch until it has returned to room temperature, otherwise incorrect test results may be obtained as a result of moisture absorption. Test strips which are not used should be placed in the plastic pouch bag again together with a desiccant, and should be preserved in a refrigerator.

Note 2: Be careful not to scratch the sample solution drop section or detecting section and do not touch them with your fingers. When handling the test strip, make sure that you hold the absorbent pad.

Note 3: Make sure that you use a sterilized pipette or chip to drop or dispense the sample solution. Change the pipette or chip for every sample solution.

Note 4: Make sure that the 100-µL portion of sample solution does not overflow the test strip when dropping it. If necessary, drop the solution in two or more portions.

Note 5: It is recommended that a wrap etc. should be placed under the test strip when dropping the sample solution.

3) Judgment of the Test Result
(Use the Reference Sheet at the end of this Instruction Manual)

(1)-1 The test results is judged as VT1 positive when a reddish purple line is observed at the VT1 test line appearance position and at the control line appearance position 15 minutes after the start of the test.

(1)-2 The test results is judged as VT2 positive when a reddish purple line is observed at the VT2 test line appearance position and at the control line appearance position 15 minutes after the start of the test.

(1)-3 The test results is judged as VT1 positive and VT2 positive when a reddish purple line is observed at the VT1 test line appearance position, the VT2 test line appearance position, and control line appearance position 15 minutes after the start of the test.

(2) Judge the test results as negative when no reddish purple line is observed at the test line appearance position, but a line is observed at the control line appearance position.

(3) Retest in cases where no reddish purple line is observed at the control appearance position, regardless of the presence or absence of a line at the test line appearance position. It is likely that there is something abnormal in the development of the sample solution in such cases.

Note 1: Samples judged as positive using this kit must be subject to identification test by other method such as PCR.

[Performance]

1) Sensitivity Test
The results of tests conducted in accordance with instructions for the preparation of the sample solution and the operating procedures for testing described in this manual will be positive when the concentration of both VT1 and VT2 is more than 2.5 ng/mL.

2) Repeatability Tests
When VT1 and/or VT2 sample solution and negative sample solutions (mEC broth with novobiocin and CAYE medium) were simultaneously tested three times each, all positive sample solutions exhibited positive results and all negative sample solutions showed negative results.

3) Minimum Detection Sensitivity
The results of testing of purified VT1 and VT2 confirmed that the minimum detection sensitivity is 2.5 ng/mL for both VT1 and VT2.

Note 1: The minimum detection sensitivity of this kit could vary depending on the effects of the components of the sample solution.

4) Cross-reactivity
(1) Cross-reactivity with the following bacterial strains has not been observed.

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli ATCC 43888, 700728, 25922, 11775</td>
<td>—</td>
</tr>
<tr>
<td>Escherichia hermannii JCM 1473</td>
<td>—</td>
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<tr>
<td>Citrobacter freundii ATCC 8090</td>
<td>—</td>
</tr>
<tr>
<td>Enterobacter aerogenes ATCC 13048</td>
<td>—</td>
</tr>
<tr>
<td>Enterobacter cloacae ATCC 13047, 49141</td>
<td>—</td>
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<tr>
<td>Enterobacter sakazukii ATCC 51329</td>
<td>—</td>
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<tr>
<td>Klebsiella oxytoca ATCC 8724</td>
<td>—</td>
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<tr>
<td>Serratia liquefaciens ATCC 27592</td>
<td>—</td>
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<tr>
<td>Serratia marcescens ATCC 8100</td>
<td>—</td>
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<tr>
<td>Serratia odorifera ATCC 33077</td>
<td>—</td>
</tr>
<tr>
<td>Proteus vulgaris ATCC 6380</td>
<td>—</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 9027</td>
<td>—</td>
</tr>
</tbody>
</table>
1) **Precautions in Handling the Kit**
   1. Read the instruction manual carefully before use. Use the kit in accordance with the test method described in this manual.
   2. This instruction manual is intended as a guideline for those in charge of testing. Verify your own operating procedures for the kit and the appropriateness of its use for each particular food.
   3. Do not use a kit whose use-by date has passed. The expiry date is indicated on the label on the external package of the kit and on the aluminum pouch of the test strip.
   4. This kit is a reagent designed to detect VT1 and VT2 in food or isolated colony. It is not be used for clinical diagnosis.
   5. Tests may give false-positive results as a result the effects of the ingredients present in the specimen and concentration of verotoxin. Positive test results from the kit should be confirmed by other test methods or procedures.
   6. Confirm with the manufacturers or distributors that any instruments and reagents (including culture media) used for preparation of sample solutions are suitable for the purpose.
   7. Product specifications may be changed without notice.

2) **Precautions Regarding Risk Prevention**
   1. Even minute amounts of Verotoxin, which the kit is designed to detect, presents strong toxicity. Infection with microorganisms such as *E. coli* may occur. Thus, pay full attention in conducting tests by wearing protective gloves and safety glasses.
   2. Tests should be performed only where appropriate equipment and facilities are available. Follow standard microorganism testing procedures under the guidance of responsible supervisors.
   3. If you accidentally get any sample solution in your eyes or mouth, adopt emergency measures, such as immediately washing away the solution with tap water, and then seek medical attention.
   4. If you feel unwell after performing a test with the kit, obtain immediate treatment from a physician.

3) **Precautions Regarding Disposal of Waste Materials**
   1. Note that surplus sample solutions and used test strips, culture media, and test samples could carry contagious microorganisms. Therefore, make sure that waste materials are subject to appropriate sterilization, for example by autoclave treatment for 20 minutes at 121 °C or immersion of the materials in a sodium chlorite solution for more than 1 hour.
   2. Discard the kits, test samples, and surplus sample solutions in strict compliance with your local waste-disposal regulations and with full consideration of environmental sanitation.

**[Storage Method and Use-By Date]**

1) Storage method: Refrigerate at 2–8 °C and shade from the light. Avoid freezing.
2) Use-by date: 12 months from the date of manufacture.

**[Packaging Unit]**

NH Immunochromato VT 1/220 tests

**[References]**