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) 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.
(3) The kit is a reagent designed to detect E. coli O157 in food. It is not to be used for clinical diagnosis.
(4) Tests may give false-positive results as a result of the effects of the ingredients present in the specimen. Positive test results from the kit should be confirmed by using the official test method or other procedures.
(5) Confirm with their manufacturers or distributors that any reagents (including culture media) required for preparation of test solutions and any other materials used are suitable for the purpose.
(6) 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.
(7) Product specifications may be changed without notice.

2) Precautions Regarding Risk Prevention
(1) Even minute amounts of E. coli O157, which the kit is designed to detect, could cause infection. For this reason, and because there is a possibility of infection by microorganisms other than E. coli O157, exercise full precautions 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 microbiom 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 test 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 test solutions in strict compliance with your local waste-disposal regulations and with full consideration of environmental sanitation.

[Storage Method and Use-By Date]

- NH Immunochromato O157
  - 20 tests (5-test × 4)
- Storage method: Refrigerate at 2–8 °C and shade from the light. Avoid freezing.
- Use-by date: 12 months from the date of manufacture.

[Packaging Unit]

| A | Test strip | 2-test × 10 packs | (1) Reagent-containing section |
| B | Instruction manual | 1 sheet |
| C | Plastic pouched bag | 1 bag |

- (2) Detecting section
  - Anti-E. coli O157 antibody (goat)
  - Anti-goat immunoglobulin antibody (rabbits)
  - Anti-E. coli O157 antibody (rabbits)

[Application]

(1) Detection of E. coli O157 in Foods
Note 1: This kit is intended to specifically detect E. coli O26 and therefore cannot detect the presence or absence of verotoxin production.

[References]


[Product Features]

[Kit contents]

1) Components**
- A: Test strip
- B: Instruction manual
- C: Plastic pouched bag

2) Ingredients**
- (1) Reagent-containing section
  - Gold colloidal labeled anti-E. coli O157 antibody (goat)
- (2) Detecting section
  - Anti-E. coli O157 antibody (rabbits)
- Anti-goat immunoglobulin antibody (rabbits)

[Application]

(1) Detection of E. coli O157 in Foods

[Reagent for food testing]

NH IMMUNOCHEMATO O157 <<<Instruction Manual>>

** Please read this manual carefully before using the kit.

[Introduction]

Food poisoning by Diarrheagenic Escherichia coli is an infectious food poisoning that is caused by the growth of E.coli taken into the intestinal tract through the ingestion of food. These Diarrheagenic E.coli can be broadly classified into five types. Among these, Enterohemorrhagic E.coli (EHEC) causes hemorrhagic colitis through the action of the verotoxin that it produces; it can also cause hemolytic uremic syndrome and encephalopathy, which can be fatal. Many cases of mass food poisoning by EHEC have occurred, so EHEC is viewed as the most dangerous among Diarrheagenic E.coli from the standpoint of food hygiene. In addition to E. coli O157, other serotypes have been detected, such as O26, O111, O118, O103, and O121. In Japan, however, most of EHEC are reported to be of E. coli O157.

This product is a kit for detecting E. coli O157 in foods by immunochromatography. Tests can be conducted rapidly and simply by means of the kit.

[Packaging Unit]

- NH Immunochromato O157
  - 20 tests (5-test × 4)

[Manufacturer]

| [Distributor and Contact] |
| listed on the label of external package |

[References]

The method for preparing a sample solution is described in accordance with the Japanese official testing method.

1) Required Equipment and Instruments
   Stomacher bag (preferably with a filter), stomacher, incubator, autoclave, culture medium, etc

2) Preparation of Test Samples
   (1) Take a test sample of more than 200g of the food under test. In cases where surface contamination is suspected, the sample is taken by scraping off 300–500cm² of the surface to a thickness of 0.2–0.3mm.
   (2) Chop and mix the whole sample collected. Weigh 25g of the sample into the stomacher bag and use this as the test specimen

3) Sample enrichment
   (1) Add 225 mL of mEC broth with novobiocin to the 25g specimen in stomacher bag and homogenize with a stomacher for 1 minute.
   (2) Incubate the specimen in the stomacher bag at 42°C for 18–24 hours.

   Note1: When commercial mEC culture medium that does not contain novobiocin is used, sterilize the medium by autoclaving and cool it. Then, sterilize a 4 mg/mL solution of novobiocin by filtration and add 5 mL of the solution to 1 L of mEC culture medium to give a final concentration of 20 mg/L.

   Note2: Instead of the mEC broth with novobiocin, it is possible to use tryptosoya broth (TSB) or growth and proliferation retardant-added TSB (mTSB, TSB-C7V, or mTSB-VCC) selective enrichment broth.

4) Sterilization
   (1) Remove the stomacher bag from the incubator after 18-24 hours. Gently mix the contents of the stomacher bag using a side-to-side motion, taking care not to splash it.
   (2) Transfer about 5 mL of the culture solution into a glass test tube by using a sterilized pipette, and sterilize the solution in an autoclave for 20 minutes at 121°C.
   (3) Remove the tube from autoclave and allow the tube to cool to room temperature to prepare the sample solution.

   Note1: The kit can also detect viable E. coli O157, but tests with a sterilized culture solution are recommended to ensure the safety of the operator.

   Note2: 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.

[Operating Procedures for Testing]

1) NH Immunochromatograph O157 Test Procedures
   (1) Bring the test strips contained in the aluminum pouch to room temperature and remove from the pouch as necessary 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 bag.**
   (3) Place the test strip carefully on the flat stand and drop a 100 µL portion of the test solution onto the test sample drop section (see the figure on the left). Otherwise, dispense a 150 µL portion of the test 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 test 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 E. coli O157 in the solution.**

   Note1: 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.**

   Note2: Be careful not to scratch the test sample drop section or expanded section and do not touch them with your hands. When handling the test strip, make sure that you hold the absorbent pad.**

   Note3: Make sure that you use a sterilized pipette or chip to drop or dispense the sample solution. Change the pipette or chip for every test solution.

   Note4: Make sure that the 150-µL portion of test solution does not overflow the test strip when dropping it. If necessary, drop the solution in two or more portions.**

   Note5: To prevent infection of the operator, it is recommended that testing be performed with the test strip. A wrap should be placed under the plate, when dropping the test solution.**

2) Judgment of the Test Results
   (1) The test results is judged as positive when a reddish purple line is observed at the test line appearance position and at the 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.

   Note1: Ensure that confirmatory tests, in accordance with the official test method or other methods, are performed on specimens that are tested positive by the kit. The proliferated and cultivated samples used for testing with the kit can be used in confirmatory tests by the official or other test method.

[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 E. coli O157 organisms is more than 1 × 10⁷ CFU/mL.*

2) Repeatability Tests
   When positive and negative test solutions of E. coli O157 were simultaneously tested three times each, all positive test solutions exhibited positive results and all negative test solutions showed negative results.

3) Minimum Detection Sensitivity
   The results of testing standard three strains of E. coli O157 and four strains of isolated bacteria confirmed that the minimum detection sensitivity is between 1 × 10⁶ and 1 × 10⁷ CFU/mL.*

   Note1: The minimum detection sensitivity of this kit could vary depending on the effects of the components of the test solution.

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

<table>
<thead>
<tr>
<th>ATCC No.</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esherichia coli</td>
<td>25922, 11775</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>13048</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>13047</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>48141</td>
</tr>
<tr>
<td>Enterobacter sakazakii</td>
<td>51329</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>8090, 43864</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>4352</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>8724</td>
</tr>
<tr>
<td>Serratia liquefaciens</td>
<td>27592</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>8106</td>
</tr>
<tr>
<td>Serratia odoriflora</td>
<td>33077</td>
</tr>
</tbody>
</table>

   (2) There is a possibility that one part of Citrobacter freundii and Salmonella Kamani (O30) will show some cross-reactivity because they contain the same antigens as E. coli O157.