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 O111 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 instruments that are 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 O111, 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 O111, 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 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 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]

(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 O111 20 tests (5-tests × 4)

[References]


[Manufacturer]

R & D Center, Nippon Meat Packers, Inc.
3-3 Midorigahara, Tsukuba, Ibaraki 300-2646, Japan
Phone:+81-29-847-7825  Fax:+81-29-847-7824
URL: http://www.rdc.nipponham.co.jp

[Reagent for food testing]

NH IMMUNOCHROMATO O111 <<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 though 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. The diarrheagenic E. coli, O111, has been detected as the second commonest serotype after O157 in foreign countries and reported to be the third commonest serotype after O157 and O26 in Japan.

This product is a kit for detecting E. coli O111 in foods by immunochromatography. Tests can be conducted rapidly and simply by means of the kit.

[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]

A: Test strip
B: Instruction manual 1 sheet
C: Plastic pouched bag 1 bag

[Application]

(1) Detection of E. coli O111 in Foods

Note 1: This kit is intended to specifically detect E. coli O111 and therefore cannot detect the presence or absence of verotoxin production.

1) Illustration of Test strip**

[Illustration of Test strip and the Principle of assay**]

a. Sample solution drop section
   (Be careful not to touch this section with your finger)

b. Reagent-containing section
c. Detecting section (Be careful not to scratch this section and touch this section with your finger)
d. Absorbent pad

f. Test line appearance position
   (Approx. 35mm from the sample solution drop section)
g. Control line appearance position
   (Approx. 33mm from the sample solution drop section)

2) Principle of assay**

When a sample solution is dropped onto the test sample drop section of the test strip, the gold colloidal-labeled anti-E. coli O111 antibody (2) in the test sample-containing section dissolves and forms complexes with E. coli O111 (1). These complexes move to the expanded section by capillary attraction and are trapped by the anti-E. coli O111 antibody (3) that is fixed in the test line appearance position. This results in the appearance of a reddish purple line of gold colloidal. This reddish purple line can be detected by visual inspection and used to judge the presence or absence of E. coli O111 in the test solution. The excess gold-labeled antibodies, regardless of the presence or absence of E. coli O111 in the test solution, travel further through the expanded section and are trapped by the anti-goat immunoglobulin antibody (4) fixed at the control line appearance position, where they form a second reddish purple line. The presence of this line indicates that the test solution has reached the expanded section.

[Precautions in Using 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 O111 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 instruments that are 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 O111, 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 O111, 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 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 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.
**Preparation of the sample Solution**
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.

Note 1: When commercial mEC culture medium that does not contain novobiocin is used, sterilize the medium by autoclaving and cool it. Then, sterilize 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.

Note 2: Instead of the mEC broth with novobiocin, it is possible to use tryptosoya broth (TSB) or growth and proliferation retardant-added TSB (mTSB, TSB-CTV, 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 the autoclave and allow the tube to cool to room temperature to prepare the sample solution.

Note 1: The kit can also detect viable E.coli O111, but tests with a sterilized culture solution are recommended to ensure the safety of the operator.

Note 2: 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) NHImmunochromat OH1 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 right). 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 O111 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 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.**

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 test solution.

Note 4: 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.**

Note 5: 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 are 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.

Note 1: 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 O111 organisms is more than 1 × 10⁶ CFU/mL.*

2) Repeatability Tests
   When positive and negative test solutions of E. coli O111 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 O111 and four strains of isolated bacteria confirmed that the minimum detection sensitivity is between 1 × 10⁴ and 1 × 10⁵ CFU/mL.*

Note 1: 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>Strains</th>
<th>ID of standard strains</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esherichia coli O157</td>
<td>ATCC22592, ATCC11775, ATCC780072, RIMD05091061</td>
<td>-</td>
</tr>
<tr>
<td>Esherichia coli O26</td>
<td>RIMD05091876, IID3005</td>
<td>-</td>
</tr>
<tr>
<td>Esherichia coli O1</td>
<td>ATCC11775</td>
<td>-</td>
</tr>
<tr>
<td>Esherichia coli O25</td>
<td>RIMD05090501</td>
<td>-</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>ATCC3090</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>ATCC13048</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>ATCC13047, ATCC40141</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacter sakazakii</td>
<td>ATCC51329</td>
<td>-</td>
</tr>
<tr>
<td>Esherichia hermanii</td>
<td>JCM1473</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>ATCC4352</td>
<td>-</td>
</tr>
<tr>
<td>Kloubaella oxytoca</td>
<td>ATCC8724</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC9027</td>
<td>-</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>ATCC6380</td>
<td>-</td>
</tr>
<tr>
<td>Serratia liquefaciens</td>
<td>ATCC27592</td>
<td>-</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>ATCC8100</td>
<td>-</td>
</tr>
<tr>
<td>Serratia oncorhinosus</td>
<td>ATCC33077</td>
<td>-</td>
</tr>
</tbody>
</table>