soleris®

E. coli

Product Number: EC-104 | SKU: 700002934

Pictured: EC-104 vial uninoculated negative (left) and inoculated positive vial (right).

Product Description and Intended Use

The *E. coli* vial (EC-104) 5.0 mL is used to detect *E. coli* species. The detection system is a peptone yeast extract base with lactose as the carbon source, and the selective agents include bile salt, sodium lauryl sulfate, and other gram-positive inhibitors. The vial has an assay time of 24 hours for most applications. Bromocresol purple (BCP) is used as the pH indicator and changes from purple to yellow as acid is produced from the *E. coli* metabolism.

In an AOAC Research Institute *Performance Tested Method*℠ (PTM) study, Soleris® for *E. coli* was found to be an effective method for detection of *E. coli* in mozzarella cheese, echinacea and cocoa powder in a dilute-to-specification format, and in pasteurized liquid egg, sweetened condensed milk, and frozen green beans in the presence/absence format.

Materials Required

1. EC-104, *E. coli* medium vials (5 mL)
2. Tryptic Soy Broth
3. Kovac's Reagent

Dependent on Sample Tested

1. Sterile 1N to 5N sodium hydroxide (NaOH) and/or hydrochloric acid (HCl)
2. pH meter or pH paper
3. Butterfield's Phosphate Buffer, 99 mL
4. Tryptone Broth

Vial Specifications

1. Vial pH is 6.7 ± 0.2
2. Vial sample capacity up to 5.0 mL

Sample Preparation

1. For AOAC PTM validated matrices, add 25 g of sample to 225 mL of Buffered Peptone Water (BPW-ISO) (for dilute-to-specification testing) or pre-warmed Tryptic Soy Broth (for presence/absence testing).
2. For all other matrices, add 11 g of sample to 99 mL of Tryptic Soy Broth.
3. If using the dilute-to-specification method, complete the dilution required.
4. If using the presence/absence method, pre-incubate the sample for 18–24 hours at 35°C.
   Note: For AOAC validated matrices, incubate the samples at 36±1°C.

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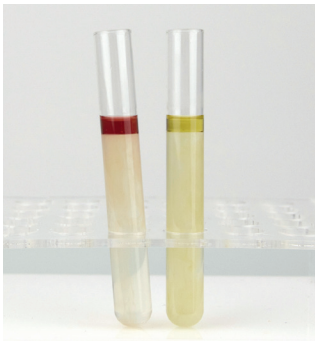

Pictured: Kovac's positive (left) and Kovac's negative (right).

Inoculation of Vial — Dilute-to-Specification

1. Inoculate the vial with up to 5.0 mL and no less than 0.10 mL of the sample to be tested. If using the dilute-to-specification method, add the volume of the appropriate dilution required.
2. Add 0–5 mL of BPB or sterile deionized water to the vial to bring the volume up to 10 mL, based on the amount of sample added to the vial
3. Cap the vial and gently invert 3 times to mix the sample. Keep cap tight.
4. Insert the vial into the Soleris® instrument set at 43.5±0.5°C and run for the preprogrammed test duration. It is not recommended to adjust the parameters without consulting Neogen® Technical Services.
5. If detection occurs, perform the Kovac's Indole confirmation test below.

Inoculation of Vial — Presence/Absence

1. Remove the sample from the incubator.
2. Transfer 0.1 mL of the incubated TSB enrichment to the EC-104 vial.
   a. For liquid egg, add 1.0 mL.
3. Add 4–5 mL of Bufferfields Phosphate Buffer to the vial to bring the volume up to 10 mL, based on the amount of sample added to the vial.
4. Cap the vial tight and invert several times to mix.
5. Insert the vial into the Soleris instrument set at 43.5± 0.5°C and run for the preprogrammed test duration. It is not recommended to adjust the parameters without consulting Neogen Technical Services.
6. If detection occurs, perform the Kovac's Indole confirmation test below.

Algorithm Utilized:

Test	Threshold	Skip	Shuteye	Test Duration	Temperature
EC-104	10	1	25	24 hours	43.5°C

Interpretation and Result

The Fusion Software will signal a positive result if the optical signal meets the algorithm criteria. Positive results will generally be detected in less than 24 h. If the Fusion software does not signal a detection during the test duration, the sample is considered negative.

E. coli Confirmation Step (Kovac's Indole)

1. Remove the EC-104 vial positive (detecting) vial from the instrument.
2. Remove 1.0 mL from the vial and add it to a test tube.
3. Add a few drops (5–8) of Kovac's reagent.
   a. Do not add Kovac's directly to the EC-104 vial, as it will destroy all viable organisms for identification.
4. Appearance of a bright pink ring at the meniscus of the broth indicates the presumptive presence of *E. coli*.
   a. If negative, the reagent layer will retain the original yellow color.
5. Presumptive Indole production positive samples should be sent for identification.

E. coli Confirmation Step (Kovac's Indole) — For Liquid Egg Sample

1. Remove the EC-104 vial positive (detecting) vial from the instrument.
2. Transfer 0.1 mL to a Soleris Tryptone Broth or peptone water tube.
3. Incubate tryptone or peptone tube 18–24 hours at 35°C.
4. Remove 0.5–1.0 mL from the Tryptone Broth and add to a test tube.
5. Add a few drops (5–8) of Kovac's reagent.
   a. Do not add Kovac's directly to the tryptone test tube, as it will destroy all viable organisms for identification.

6. Appearance of a bright pink ring at the meniscus of the broth indicates the presumptive presence of *E. coli*.
    a. If negative, the reagent layer will retain the original yellow color.
7. Presumptive Indole production positive samples should be sent for identification.

Disclaimers:

Information provided is based on validation procedures that NEOGEN performed in NEOGEN laboratories. Deviation from procedures is possible, but should be discussed with NEOGEN Technical Services.

Appearance of the vials should be inspected prior to use.

If shuteye detections are observed, the threshold may need to be adjusted based on the product matrix. Certain product matrices may require parameter adjustments, including increased test duration. For more information, contact Neogen Technical Services at 517.372.9200 or visit our website at Neogen.com.

Reference the Soleris Operating Manual for troubleshooting, use of instrument, and interpretation of results.

Safety Precautions

Use of this test should be restricted to individuals with appropriate laboratory training in microbiology as some *E. coli* are potentially pathogenic. Reagents are for laboratory use only. All pipetting transfers must be made using either a disposable pipet and pipetting aid or micropipettor with disposable tips. Culture media contains antimicrobial selective agents and dyes. Wear appropriate PPE and avoid contact with skin and mucous membranes. Refer to the Safety Data Sheet available from NEOGEN for more information. Used Soleris vials should be handled and disposed of as potentially infectious material. The preferred method for disposal of contaminated materials, including used vials, sample homogenates, pipettes, etc., is autoclaving. Items that cannot be autoclaved may be decontaminated by using a disinfectant solution, e.g., 10% household bleach, followed by rinsing with water. Consult with your facility safety director for specific instructions.

Neogen Corporation, 620 Lesher Place, Lansing, MI 48912 USA.