soleris®

E. coli

Product Number: EC-104

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

Introduction

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. The 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℠ study, Soleris® for *E. coli* was found to be an effective method for detection of *E. coli* in the following sample types: cocoa powder, echinacea, mozzarella cheese, pasteurized liquid egg, condensed milk, and frozen green beans.

Materials Required

1. EC-104, *E. coli* medium vials (5 mL)
2. TS-124, Tryptic Soy Broth, 100 mL or BLX-TSB90, Tryptic Soy Broth, 90 mL
3. Kovac® Reagent (KO-122)

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 (BPB-99)
4. Tryptone Broth (TT-123)

Vial Specifications

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

Sample Preparation

1. Add 10 g of sample to 100 mL of Tryptic Soy Broth (TS-124), or 90 mL of Tryptic Soy Broth (BLX-TSB90).
2. If using the dilute-to-specification method, complete the dilution required.
3. If using the presence/absence method, take the sample and incubate for 18–24 hours at 35°C.

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 you use dilute-to-specification method, add the volume of the appropriate dilution required.
2. Cap the vial and gently invert 3 times to mix the sample. Keep cap tight.
3. Insert the vial into the Soleris® instrument set at 43.5°C and run for the preprogrammed test duration. It is not recommended to adjust the parameters without consulting NEOGEN® Technical Services.

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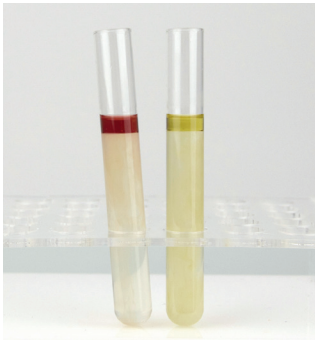

Pictured: Kovac's positive (left) and Kovac's negative (right).

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 9 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°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 Indole production test.

Algorithm Utilized:

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

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 (KO-122)
   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 positive samples should be sent out 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 (KO-122).
   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.*
7. If negative, the reagent layer will retain the original yellow color.
8. Presumptive positive samples should be sent out 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.

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

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