



# soleris

## *E. coli*

Product Number: EC-104



Pictured: EC-104 vial uninoculated (left) and inoculated 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 18–24 hours for most applications. Acidification of the medium due to lactose utilization changes the pH. A more concentrated inhibitory system and 44.5°C temperature add to the medium's selectivity. Bromocresol purple (BCP) is used as the pH indicator. The BCP changes from purple to yellow as acid is produced from *E. coli* metabolism.

In an AOAC Research Institute Performance Tested Method (SM) 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

### 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)

### Vial Specifications

1. Vial pH is  $6.7 \pm 0.2$
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).
2. Adjust the pH of the dilution to  $6.5 \pm 0.5$ .
3. Incubate the dilution for 18–24 hours at 35°C.

### Inoculation of Vial

1. Transfer 0.1 mL of the incubated TSB enrichment to the EC-104 vial.
2. Add 4.5 mL BPB to the vial.
3. Cap the vial tight and invert several times to mix.
4. Insert the vial into the Soleris® instrument set at 44.5°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 confirmation test.



### Algorithm Utilized:

Test	Threshold	Skip	Shuteye	Test Duration	Temperature
EC-104	10	1	25	18–24 hours	44.5°C

### *E. coli* Confirmation Step (Indole)

1. Remove the EC-104 vial positive (detecting) vial from the instrument.
2. Transfer 0.1 mL to a Seleris Tryptone Broth tube.
3. Incubate tryptone 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.



### Test Methods *E. coli* Confirmation Procedure

1. From a positive Seleris *E. coli* medium vial, invert vial gently and pipette 0.1 mL of the broth medium into a Seleris Tryptone Broth tube and onto Tryptic Soy Agar (TSA).
2. Streak the TSA plate for isolation using a 10 µL inoculation loop and place 1 coli complete disk onto the lawn of the streak.
3. Incubate tryptone tube 18–24 hours at 35°C.
4. Incubate TSA plate with disk at 35°C for 30–38 hours.

### *E. coli* Results Interpretation

1. After incubation, add 2–3 drops of Kovac's Reagent to the Seleris Tryptone Broth tube.
2. Appearance of a bright pink ring at the meniscus of the broth indicates possible presence of *E. coli*.
3. After incubation, visually observe the appearance of the coli complete disk on TSA. The presence of a blue-green color indicates a positive coliform result.
4. When viewed under a UV light, the disk will fluoresce if *E. coli* is present.
5. Positive results by both methods is sufficient to confirm presence of *E. coli* in the sample.

### 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 Seleris Operating Manual for troubleshooting, use of instrument, and interpretation of results.

