



Soleris® vial uninoculated (left) and inoculated vial (right).

The *E. coli* Vial (S2-EC) is used to detect *Escherichia coli* species. The S2-EC vial contains a broth enrichment medium with a fluorescent indicator. The vial has an assay time of 18 hours for most applications. The reading frame contains a soft agar plug which reduces product interference with the detection of microbial growth. As *E. coli* organism grows in the broth medium, the utilization of MUG (4-methylumbelliferyl-beta-D-glucuronide) by *E. coli* (enzymatic reaction) produced diffuses into the soft agar plug. The fluorescent end product called methylumbelliferone is read by the optical sensors in the instrument.

### Materials Required:

1. S2-EC, *E. coli* (S2-EC) vial
2. Tryptic Soy broth (BLX-TSB90)
  - a. If required, use a designated neutralization broth, such as D/E Neutralizer, TAT Broth, Modified Lethen Broth, etc.
3. Soleris *E. coli* MUG supplement (S2-ECM)
4. Kovac's Indole reagent (KO-122)
5. Test tube for confirmation

### Dependent on Sample Tested:

1. Sterile 1 N to 5N sodium hydroxide (NaOH) and/or hydrochloric acid (HCl)
2. pH meter or pH paper

### Vial Specifications

1. Vial pH is  $7.2 \pm 0.2$
2. Vial sample capacity: 0.1 mL

### Sample Preparation

1. For USP testing, perform 1:10 dilution by adding 10 g of sample in 90 mL of Tryptic Soy Broth (See Neogen Rapid Microbiology System Validation Book, Introduction, p.5) or designated neutralization broth.
  - a. Check pH and adjust, if necessary, to  $7.0 \pm 1.0$ .
2. Incubate for 18–24 hours at 35°C.

### Inoculation of Vial

1. Transfer 0.12 mL of the Soleris *E. coli* MUG Supplement (S2-ECM) to the S2-EC vial.
2. Cap the vial and gently invert 3 times to mix sample.
3. Transfer 0.1 mL of the incubated enrichment to the S2-EC vial.
4. Cap the vial and gently invert 3 times to mix sample. Keep cap tight.
5. Insert the vial into the BioLumix® instrument set at 35°C and run for the pre-programmed 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.

### Algorithm Utilized:

Test	Test Type	Detection Level	Resolution	Ignore	Test Duration	Temp
S2-EC	Fluorescence	40	2	20	18 hours	35°C

### *E. coli* Confirmation Step (Kovac's Indole)

1. Remove the S2-EC vial positive (detecting) vial from the instrument.
2. Transfer 0.5 – 1.0 mL 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 S2-EC 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.



### 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.

Samples may need to be pH adjusted for all vials.

Appearance of the vials should be inspected prior to use.

Certain product matrices may require parameter adjustments, including increased test duration. For more information contact Neogen Technical Services.