



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

The *Salmonella* (S2-SAL) Vial (9 mL) is a screening vial for *Salmonella* species in nutraceutical products. The vial is CO₂ vial. Following a *Salmonella* selective enrichment protocol, organisms grow in the vial broth medium. The vial has an assay time of 20 hours for most applications. As organisms grow in the broth medium, the carbon dioxide (CO₂) produced diffuses through a membrane layer into a soft agar plug containing a dye indicator. The membrane layer also serves as a barrier, eliminating product interference with the reading window. The CO₂ released during the organism growth changes the agar plug from green/blue-green to yellow. The color change in the dye is read by the instrument.

Materials Required:

1. S2-SAL, *Salmonella* (S2-SAL) vial
2. For USP: 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. Rappaport-Vassiliadis *Salmonella* Supplement (BLX-RVS)
4. BLX *Salmonella* Supplement (S2-SALI)
5. Reveal 2.0 for *Salmonella* (PN 9706)

Dependent on Sample Tested:

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

Vial Specifications

1. Vial pH is 7.0 ± 0.2
2. Vial sample capacity up to 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 ± 1.0.
2. Incubate for 18–24 hours at 35°C.
3. Transfer 1.0 mL of the incubated TSB enrichment to the Rappaport-Vassiliadis *Salmonella* Broth (BLX-RVS) and incubate for 6–8 hours at 35°C.
 - a. Samples that have a high amount of background can be incubated at 42°C for 6–8 hours.

Vial Preparation

1. Remove S2-SAL vials from the refrigerator and allow to equilibrate to room temperature.

Inoculation of Vial

1. Transfer 0.1 mL of the S2 *Salmonella* Supplement (S2-SALI) to the S2-SAL vial.
2. Cap the vial and gently invert 3 times to mix sample.
3. Transfer 0.1 mL of the incubated Rappaport-Vassiliadis *Salmonella* Supplement to the S2-SAL vial.
4. Cap the vial and gently invert 3 times to mix sample. Keep cap tight.
5. Insert the vial into the Soleris 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 Reveal 2.0 for *Salmonella* test.

Algorithm Utilized:

Test	Threshold	Skip	Shuteye	Duration	Temperature
S2-SAL	8	1	25	20 hours	35°C

Salmonella Confirmation Step

1. Remove the S2-SAL vial positive (detecting) vial from the instrument.
2. Transfer 0.20 mL or 8 drops to the Reveal sample cup.
3. Remove the required number of Reveal 2.0 for *Salmonella* test devices from the container.
4. Place Reveal device with sample arrows facing down into the sample cup containing the sample and incubate at ambient temperature for 15 minutes.
5. Record Reveal results after 15 minutes.
 - a. Line in both control and test zone in 15 minutes is considered presumptive positive.
 - b. Line in control zone only at 15 minutes is considered negative.
 - c. If no line appears in the control zone, the test is considered invalid and the sample should be retested with another device.

NOTE: Results after 20 minutes will be invalid due to overdevelopment of the device.
6. 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.

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.