

Specification

Selective supplement for the isolation of *Salmonella*

Presentation

10 Freeze dried vials
Vial
with: 6 ± 0.1 g

Packaging Details

23x50 mm glass vials, tag labelled, White plastic cap - 10 vials per box.

Shelf Life

49 months

Storage

2-8 °C

Composition

Composition (g/vial)

Antibiotic Mix..... 0.0085

Reconstitute the original freeze-dried vial by adding

Sterile Distilled Water..... 5 ml

Note: Each vial is sufficient to supplement 500 ml of Salmonella Chromogenic Agar (Cat. 1122)

Description /Technique

Description:

Salmonella Chromogenic Supplement contains a mixture of antibiotics that inhibit the accompanying flora to avoid false positives. This supplement is added to the Salmonella Chromogenic Agar (Cat. 1122).

Salmonella Chromogenic Agar is a selective chromogenic medium, used for the detection and presumptive identification of *Salmonella* species from clinical samples, foods and waters. The media traditionally used to differentiate species of *Salmonella* from the rest of the Enterobacteriaceae family, based on their capacity to produce hydrogen sulfide and their inability to ferment lactose, are not really adequate as there are more than 2.000 species of *Salmonella* which do not have these characteristics.

To identify *Salmonella* species, this medium contains a chromogenic agent based on the combination of two chromogenic substrates that ease quick identification. Magenta colonies are a result of the hydrolysis of one of the chromogenic substrate by the *Salmonella* species due to the inability to use another chromogenic substrate. Microorganisms producing the enzyme that cleaves the second chromogenic substrate will produce blue-green colonies. Thus, non-Salmonella organisms appear blue-green or are not stained by any of the chromogenes of the medium. Supplement is added when more selectivity is desired, as it inhibits the accompanying flora, specially *Pseudomonas*, that could appear in the same color as Salmonella colonies.

The medium can be used as a secondary medium for the detection of *Salmonella* in food and water according to ISO 6579 and ISO 19250 respectively.

Technique:

Aseptically reconstitute 1 vial with 5 ml of sterile distilled water. Mix gently until complete dissolution and aseptically add to 500 ml of Salmonella Chromogenic Agar (Cat. 1122), previously cooled to 50 °C. Mix well and distribute into sterile containers.

Instructions for use:

» For clinical diagnosis, the type of sample is fecal and from rectal tract.

- Inoculate the sample on the surface of the Salmonella Chromogenic Agar plates, streaking to obtain isolated colonies. - Incubate at a temperature of 35 ± 2 °C for 18-24 hours.

- Examine the color of the colonies.

» For other uses not covered by the CE marking:

Detection of *Salmonella* spp in foods according to ISO 6579:

- Preenrichment in non-selective liquid medium:

Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 34-38 °C for 18 h.

- Enrichment in/on selective media:

Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) or the Modified Semisolid Rappaport Vassiliadis medium (MSRV) (Cat. 1376), and the Tetrathionate Broth (Muller-Kauffmann) (Cat. 1173). The Rappaport Soy Broth and the Modified Semisolid Rappaport medium are incubated at 41,5 °C for 24 h, and the Tetrathionate Broth at 37 °C for 24 h.

- Plating out on selective solid media:

From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar, in this case, Salmonella Chromogenic Agar (Cat. 1122).

Incubate the XLD plates inverted at 35 ± 2 °C for 18-24 h. Incubate the Salmonella Chromogenic Agar (Cat. 1122) at 35 ± 2 °C for 18-24 hours.

- Confirmation:

Subculture colonies of presumptive *Salmonella* and confirm their identity by biochemicals and serological tests.

Detection of *Salmonella* spp. in water samples according to ISO 19250:

- Preenrichment in non-selective medium:

Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 36 ± 2 °C for 18 ± 2 h.

- Enrichment in selective media:

Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) and the Tetrathionate Broth (Muller-Kauffmann) (Cat. 1173).

The Rappaport Soy Broth is incubated at $41,5 \pm 1$ °C and the Tetrathionate Broth at 37 ± 1 °C, both of them for 24 ± 3 hours.

- Plating out on selective solid media:

From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar in this case, (Salmonella Chromogenic Agar (Cat. 1122).

Incubate the XLD plates inverted at 35 ± 2 °C for 18-24 h. Incubate the Salmonella Chromogenic Agar (Cat. 1122) at 35 ± 2 °C for 18-24 hours.

- Confirmation:

Subculture colonies of presumptive *Salmonella* and confirm their identity by biochemicals and serological tests.

Quality control**Physical/Chemical control**

Color: Off-white

pH: at 25°C

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Distribute the complete medium, cooled to 50 °C, into 90 mm plates

Aerobiosis. Incubation at 35 ± 2 °C, reading after 18-24 hours.

Microorganism*Escherichia coli* ATCC® 25922, WDCM 00013*Salmonella enterica* ATCC® 13076, WDCM 00030*Salmonella typhimurium* ATCC® 14028, WDCM 00031*Proteus hauseri* ATCC® 13315**Sterility Control**

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

Growth

Partial inhibition - Green to blue colonies

Good - Magenta

Good - Magenta

Good - Colourless

Bibliography

Ryan N. (1985) Personal communication.

Rogol M., Sechter I., Grinberg L., Gerichter Ch. B. (1992) J. Med. Microbiol. 12. 229-231.