

**INSTRUCTIONS FOR USE****ChromArt****SALMONELLA SELECTIVE SUPPLEMENT**

Freeze-dried and liquid selective supplements

**1 - INTENDED USE**

*In vitro* diagnostics. Selective supplement to be added to Chromogenic Salmonella Agar Base, for the isolation and differentiation of *Salmonella* spp., from clinical and non-clinical specimens.

**2 - COMPOSITION****Salmonella Selective Supplement Vial A****Vial contents for 500 ml of medium**

Emulsifying agents 5.7 mL

**Salmonella Selective Supplement Vial B****Vial contents for 500 ml of medium**

Cefsulodin 2.5 mg

**3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE**

Salmonella Selective Supplement Vial A and Vial B, added to Chromogenic Salmonella Agar Base, can be used for the preparation of a complete medium for the isolation and differentiation of *Salmonella* spp., from clinical and non-clinical specimens.

Differentiation of *Salmonella* from other organisms that grow is achieved by:

- a chromogenic substrate for  $C_{\beta}$  esterase enzyme, that is cleaved by *Salmonella* spp. with the release of an insoluble magenta-red chromophore.
  - a chromogenic glucopyranoside derivative which is cleaved by  $\beta$ -glucosidase with the release of an insoluble blue-green chromophore.
- The selectivity of the medium is due to the presence of cefsulodin, a third generation cephalosporin antibiotic that has very specific activity against *P. aeruginosa* and *S. aureus*, sodium desoxycholate that suppresses the growth of Gram-positive and some Gram-negative bacteria and Tergitol 4, active mainly against the growth of *Proteus* spp.

**4- DIRECTIONS FOR MEDIA PREPARATION**

Salmonella Selective Supplement Vial A is ready to use.

Dissolve the contents of one vial of Salmonella Selective Supplement Vial B with 2 mL of sterile purified water.

Prepare 500 mL of Chromogenic Salmonella Agar Base (REF 405350), autoclaved and cooled to 47-50°C and add the contents of one vial of Salmonella Selective Supplement Vial A and of one vial of reconstituted Salmonella Selective Supplement Vial B, under aseptic conditions. Mix well and distribute into sterile Petri dishes.

**5 - PHYSICAL CHARACTERISTICS****SALMONELLA SELECTIVE SUPPLEMENT VIAL A**

Appearance of solution pale yellow slightly opalescent

**SALMONELLA SELECTIVE SUPPLEMENT VIAL B**

Appearance of lyophilised pellet short dense pastille

Appearance of solution colourless limpid

**6 - MATERIALS PROVIDED – PACKAGING**

Product	Type	REF	Pack
Salmonella Selective Supplement	Selective supplement	4240013	5 + 5 vials, each for 500 mL of medium CND: W01040101045; EDMA: 14.01.01.04; RDM:1892762/R

**7 - MATERIALS REQUIRED BUT NOT PROVIDED**

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

**8 - SPECIMENS**

Chromogenic Salmonella Agar Base added with Salmonella Selective Supplements is intended for the bacteriological processing of clinical specimens such as faeces and rectal swabs. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.<sup>1</sup> Consult appropriate standard methods for details of collection and preparation of non-clinical specimens.<sup>2,3</sup>

**9- TEST PROCEDURE**

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment step in Selenite Broth followed by sub-culture to Chromogenic Salmonella Agar and to a second plating medium.

Incubate inoculated plates with the specimen or with specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours.

Consult appropriate references for the detection of *Salmonella* in food and water.<sup>2,3</sup>

**10 - READING AND INTERPRETATION**

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Interpretation of colonies' colours:





Microorganism	Growth characteristics
<i>Salmonella</i> spp.	good growth, magenta-red colonies
<i>Salmonella</i> spp. lac+	good growth, magenta-red colonies
<i>Salmonella</i> Typhi	good growth, magenta-red colonies
<i>E. coli</i>	poor growth with colourless colonies
<i>Enterobacter</i> spp.	growth with blue-green colonies
<i>Klebsiella</i> spp.	poor growth with blue-green colonies
<i>Pseudomonas</i> spp.	inhibited
<i>Proteus</i> spp.	poor growth with pale brown or green colonies
Gram-positive bacteria	inhibited

### 11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
S.Typhimurium ATCC 14028	35-37°C / 18-24h / A	growth, magenta-red colonies
S.Enteritidis ATCC 13076	35-37°C / 18-24h / A	growth, magenta-red colonies
<i>E.aerogenes</i> ATCC 13048	35-37°C / 18-24h / A	growth, blue-green colonies
<i>P.aeruginosa</i> ATCC 27853	35-37°C / 18-24h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

### 12 - PERFORMANCES CHARACTERISTICS

Chromogenic Salmonella Agar was evaluated by Babic-Ergeg et al.<sup>4</sup> on 3,000 stool specimens, 45 of which positive for *Salmonella*, including SS Agar as the reference medium. The authors reported a sensitivity of 100% and a specificity of 99% in the isolation and preliminary identification of *Salmonella* colonies.

In another independent study<sup>5</sup>, 50 pure cultures of *Salmonella* of clinical origin, gave all the specific chromatic reactions; among the other 80 strains of Gram-negative bacteria tested, not belonging to the *Salmonella* genus, 3 out of 3 strains of *P.aeruginosa* and 1 out of 3 strain of *A.baumannii* provided chromatic results similar to *Salmonella* spp. (red-pink colonies), the remaining 76 strains of *Enterobacteriaceae* gave non-typical chromatic reactions; 20 out of 20 strains of Gram-positive bacteria were totally inhibited.

Chromogenic Salmonella Agar performance was evaluated with an in-house study, compared to Hektoen Enteric Agar (HEA). Productivity, selectivity and specificity have been evaluated using 43 bacterial strains: 8 target strains and 35 non target strains. 8 *Salmonella* strains, including 2 *S.Typhi*, showed a good growth with magenta-red colonies; 3 *Shigella* strains showed a poorer growth than on HEA with colourless colonies; 22 *Enterobacteriaceae* strains belonging to 9 genera showed a poorer growth than on HEA with colourless or blue-green colonies; 4 *P.aeruginosa* strains were totally inhibited; 2 non-fermenters strains were totally inhibited and *A.hydrophila* grew with magenta red colonies; 1 Gram-positive strain was totally inhibited and 1 yeast strain was partially inhibited showing colourless colonies.

Prior to release for sale a representative sample of all lots of dehydrated Chromogenic Salmonella Agar supplemented with Salmonella Selective Supplement is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by semi-quantitative ecometric technique with the target strains *S.Enteritidis* ATCC 13076, *S.Typhimurium* ATCC 14028 and *S.arizonae* clinical isolate. After incubation at 35-37°C for 18-24 hours, colonies' colour and the amount of growth is evaluated and recorded.

Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10<sup>-1</sup> to 10<sup>-6</sup> of a 0.5 McFarland suspension of the non-target strains *E.faecalis* ATCC 19433, *S.aureus* ATCC 25923, *E.coli* ATCC 8739, *P.vulgaris* ATCC 13315, *A.calcoaceticus* ATCC 19606, *P.aeruginosa* ATCC 27853, *A.hydrophila* ATCC 7965, *Mucor* sp, environmental isolate. The growth of non-target strains *E.faecalis*, *S.aureus*, *Mucor* sp, *P.aeruginosa*, is inhibited at the dilution 10<sup>-1</sup>, the growth of *P.vulgaris*, *A.calcoaceticus*, *A.hydrophila* is partially inhibited. According to the specifications, the non-target strains colonies show typical blue-green colour or are colourless. Only *A.hydrophila* grows slightly with magenta-red colonies.

### 13 - LIMITATIONS OF THE METHOD

- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella*, with lower selectivity such as Mac Conkey Agar should be used; other media for the isolation of other enteric pathogens should be inoculated with the specimen.
- Some strains of *Pseudomonas*, *Acinetobacter* and *Aeromonas*, resistant to antimicrobial agents of the medium, may grow with red-pink colonies, differentiable from *Salmonella* with oxidase test.
- The growth rate on the plates also depends on the nutritional requirements of *Salmonella*. It is possible that some strains with particular metabolic characteristics may not grow on the medium or grow colourless (e.g., *Salmonella enterica* serovar Dublin grows with white colonies).
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This selective supplement and the medium base are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

### 14 - PRECAUTIONS AND WARNINGS

- Salmonella Selective Supplement is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Salmonella Selective Supplement is not classified as dangerous according to current European legislation.
- The supplement and the medium base shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the preparation process of plated media.
- Salmonella Selective Supplement Vial B is sterilized by membrane filtration.





- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use Salmonella Selective Supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it).
- Notify Biolife Italiana Srl ([complaint@biolifeitaliana.it](mailto:complaint@biolifeitaliana.it)) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

### 15 - STORAGE CONDITIONS AND SHELF LIFE

#### Salmonella Selective Supplement Vial A

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened the product should be used immediately. During storage some needles or crystals can be formed. They dissolve with slight agitation after returning the reagent to room temperature. This characteristic does not affect the performance of the product.

#### Salmonella Selective Supplement Vial B

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of plated media and the validation of their shelf life, according to the applied storage conditions (temperature and packaging).

### 16 - REFERENCES

1. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
2. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella
3. ISO 19250:2010 Water quality — Determination of Salmonella species
4. Babic-Erceg A et al. 12th European Congress of Clinical Microbiology and Infectious Diseases. Milan, April 24-27, 2002
5. Andreoni S. et al. Microbiologia Medica, 2002.

## 4240013A SALMONELLA SELECTIVE SUPPLEMENT VIAL A

SDS rev 2

Regulation (EU) 2020/878

**Contains:** Sodium tetradecyl sulfate

### Classification

Skin corrosion, category 1B H314 Causes severe skin burns and eye damage.  
Serious eye damage, category 1 H318 Causes serious eye damage.

### Labelling

Hazard pictograms:



### Signal words **Danger**

Hazard statements:

H314 Causes severe skin burns and eye damage.

Precautionary statements:

P260 Do not breathe dust / fume / gas / mist / vapours / spray.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].

P280 Wear protective gloves/ protective clothing / eye protection / face protection.

P310 Immediately call a POISON CENTER / doctor / . . .

P264 Wash . . . thoroughly after handling.

## 4240013B SALMONELLA SELECTIVE SUPPLEMENT VIAL B

SDS rev 2

Regulation (EU) 2020/878





**Contains : cefsulodin**

**Classification**

Respiratory sensitization, category 1 H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Skin sensitization, category 1 H317 May cause an allergic skin reaction.

**Labelling**

Hazard pictograms:



**Signal words**      **Danger**

Hazard statements:

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

H317 May cause an allergic skin reaction.

Precautionary statements:

P261 Avoid breathing dust / fume / gas / mist / vapours / spray.

P280 Wear protective gloves.

P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER / doctor / . . .

P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.

P333+P313 If skin irritation or rash occurs: Get medical advice / attention.

**TABLE OF APPLICABLE SYMBOLS**

<b>REF</b> or REF Catalogue number	<b>LOT</b> Batch code	<b>IVD</b> <i>In vitro</i> Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

**REVISION HISTORY**

Version	Description of changes	Date
Revision 1	Updated layout and contents	2022/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

