

ChromArt

CHROMOGENIC SALMONELLA AGAR BASE II SALMONELLA SELECTIVE SUPPLEMENT II CHROMOGENIC SALMONELLA AGAR II

Dehydrated culture medium, supplements and ready to use plates

1 - INTENDED USE

Selective and chromogenic medium for the isolation and differentiation of *Salmonella* spp.

2 - COMPOSITIONS***CHROMOGENIC SALMONELLA AGAR BASE II
DEHYDRATED MEDIUM.****TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER***

| | |
|--|--------|
| Peptones | 10.0 g |
| Selective compounds, organic and inorganic salts | 12.0 g |
| Chromogenic mix | 0.9 g |
| Agar | 15.0 g |

SALMONELLA SELECTIVE SUPPLEMENTS II**SALMONELLA SELECTIVE SUPPLEMENT II VIAL A****VIAL CONTENTS FOR 500 ML OF MEDIUM**

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|--------------------|--------|
| Emulsifying agents | 5.0 mL |
|--------------------|--------|

SALMONELLA SELECTIVE SUPPLEMENT II VIAL B**VIAL CONTENTS FOR 500 ML OF MEDIUM**

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|--|--------|
| Antibiotic mix (cefsulodin, novobiocin, linezolid) | 8.5 mg |
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CHROMOGENIC SALMONELLA AGAR II**READY-TO-USE PLATES - TYPICAL FORMULA**

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|--|---------|
| Peptones | 10.0 g |
| Selective compounds, organic and inorganic salts | 12.0 g |
| Chromogenic mix | 0.9 g |
| Agar | 15.0 g |
| Opacifier | 10.0 g |
| Emulsifying agents | 10.0 mL |
| Antibiotic mix (cefsulodin, novobiocin, linezolid) | 17.0 mg |
| Purified water | 1000 mL |

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Salmonella remains one of the most important causes of foodborne gastroenteritis. For at least 30 years, fluorogenic and chromogenic tests and specific culture media have been available for the detection of *Salmonella* spp.

In 1987, Biolife Italiana developed and introduced a rapid fluorogenic screening reagent (MUCAP Test) for the identification of *Salmonella* colonies, based on the detection of a *Salmonella*-specific enzyme, C8 esterase, using a fluorogenic substrate conjugated with 4-methylumbelliferone.¹ Some years later, the same principle of C8 esterase detection was exploited for the development of a chromogenic culture medium, Chromogenic Salmonella Agar, which demonstrated high specificity and sensitivity for the detection of *Salmonella* spp.^{2,3}

Chromogenic Salmonella Agar II is an evolution of Chromogenic Salmonella Agar, designed to improve its selective and differential properties and the transparency of the medium base.

Chromogenic Salmonella Agar II is a selective and differential medium with a clear background, suitable for the isolation of *Salmonella* spp. and for the presumptive identification of colonies. Chromogenic media are included as a second culture medium in ISO standards for the detection of *Salmonella* in food and water.^{5,6}

Peptones provide carbon, nitrogen, vitamins and trace elements for bacterial growth. The selective compounds incorporated in the medium are as follows: cefsulodin, a third-generation cephalosporin antibiotic with highly specific activity against *P. aeruginosa* and *S. aureus*; novobiocin, linezolid and sodium deoxycholate, which inhibit the growth of Gram-positive and some Gram-negative bacteria. The contents of vial A are used to emulsify the ingredients of the culture medium.

Differentiation of *Salmonella* from other growing organisms is achieved by means of:

- a chromogenic substrate for the C8 esterase enzyme, which is cleaved by *Salmonella* spp. with the release of an insoluble magenta-red chromophore;
- a chromogenic glucopyranoside derivative, which is cleaved by β -glucosidase with the release of an insoluble blue-green chromophore.

Some *Enterobacteriaceae*, including *Klebsiella* and *Enterobacter*, but not *Salmonella*, are β -glucosidase-positive and, if growing, form blue-green or dark blue colonies, even though they are esterase-positive, making them easy to differentiate from the magenta-red *Salmonella* colonies. The chromogenic and selective compounds in the medium also allow the detection of rare lactose-positive *Salmonella* strains, which are not detected on traditional media based on lactose fermentation. Chromogenic Salmonella Agar II is also useful for the detection of *S. Typhi* and *S. Paratyphi*. As the opacifying agent is omitted from the dehydrated formulation, the prepared plates have a transparent background. Ready-to-use plates contain silica; therefore, the medium appears uniformly opaque, which improves the visual differentiation of colony colours.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 19 g in 500 mL of cold purified water; add the content of one vial of Salmonella Selective Supplement Vial A, heat to boiling stirring constantly and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of Salmonella Selective Supplement Vial B, reconstituted with 2 mL of sterile purified water. Mix well and pour into sterile Petri dishes. To prepare plates with an opaque background, add 10-15 g/L of silica to the base medium before autoclaving.





Specificity is tested by semi-quantitative ecometric technique with the non-target strain *K. pneumoniae* ATCC 700603 which, after incubation at 35-37°C for 18-24 hours, grows with green -blue colonies.

Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10⁻¹ to 10⁻⁶ of a 0.5 McFarland suspension of the non-target strains *E. faecalis* ATCC 19433, *E. coli* ATCC 25922, *P. vulgaris* ATCC 13315, *A. calcoaceticus* ATCC 19606, *P. aeruginosa* ATCC 27853, *A. hydrophila* ATCC 7966, *Mucor* sp environmental isolate. The growth of non-target strains *E. faecalis*, *P. aeruginosa*, *A. calcoaceticus*, *A. hydrophila* and *Mucor* is inhibited at the dilution 10⁻¹, the growth of *E. coli* and *P. vulgaris* is partially inhibited.

According to the specifications, the non-target strains colonies show typical blue-green colour or are colourless.

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*, such as XLD Agar should be used.
- 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 depends on the nutritional requirements of *Salmonella* and on the resistance to the antimicrobials present. It is possible that some strains with particular metabolic characteristics may not grow or grow colourless (e.g., *Salmonella enterica* serovar Dublin grows with white colonies).
- Appropriate tests are required for complete identification and epidemiological typing of colonies.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement must be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before the use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. Therefore, it is recommended that the ready-to use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented for the risk reduction linked to infectious animal diseases.
- The freeze-dried supplement is sterilized by membrane sterilisation; the liquid supplement undergoes autoclave sterilisation.
- Be careful when opening the metal ring of the vials to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium, supplements and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production material 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Salmonella Selective Supplement II 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 II 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).

Ready to use plates

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).














16 - REFERENCES

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2. Babic-Erceg A et al. 12th European Congress of Clinical Microbiology and Infectious Diseases. Milan, April 24-27, 2002
3. Andreoni S. et al. *Microbiologia Medica*, 2002.
4. Istituto Superiore di Sanità. Le infezioni da *Salmonella*: diagnostica, epidemiologia e sorveglianza. Caterina Graziani, Pasquale Galetta, Luca Busani, Anna Maria Dionisi, Emma Filetici, Antonia Ricci, Alfredo Caprioli, Ida Luzzi 2005, 49 p. Rapporti ISTISAN 05/27
5. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella*
6. ISO19250:2010 Water quality — Determination of *Salmonella* species
7. 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.





TABLE OF APPLICABLE SYMBOLS

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|--|--|--|--|---|---|
|  or  Catalogue number |  Batch code |  Manufacturer |  This side up |  Store in a dry place |  Fragile |
|  Temperature limitation |  Content sufficient for <n> tests |  Consult Instructions for Use |  Use by |  Keep away from direct light |  For single use only |

REVISION HISTORY

| Version | Description of changes | Date |
|------------|----------------------------------|---------|
| Revision 0 | First edition | 2025/12 |
| Revision 1 | Update of quality control method | 2026/02 |

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

