. 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

Emulsifying agents	5.0 mL

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

Antibiotic mix (cefsulodin, novobiocin, linezolid)	8.5 mg

CHROMOGENIC SALMONELLA AGAR II
READY-TO-USE PLATES - TYPICAL FORMULA

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.

5 - PHYSICAL CHARACTERISTICS

CHROMOGENIC SALMONELLA AGAR BASE II	
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	light yellowish, limpid
Final pH at 20-25 °C	7.2 ± 0.2
SALMONELLA SELECTIVE SUPPLEMENT II VIAL A	
Appearance of solution	pale yellow slightly opalescent
SALMONELLA SELECTIVE SUPPLEMENT II VIAL B	
Appearance of lyophilised pellet	short dense pastille
Appearance of solution	colourless limpid
CHROMOGENIC SALMONELLA AGAR II	
Ready-to-use plates appearance	whitish, opaque

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Chromogenic Salmonella Agar Base II	Dehydrated medium	405350N2	500 g (13,2 L)
Chromogenic Salmonella Agar Base II	Dehydrated medium	405350N4	5 kg (132 L)
Salmonella Selective Supplement II	Selective supplement	4240013N	5 vials A+ 5 vials B, each for 500 mL of medium
Chromogenic Salmonella Agar II	Ready-to-use plates	545350N	20 plates

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 II is intended for the bacteriological processing of food and environmental specimens. Good laboratory practices for collection, transport and storage of specimens should be applied.[7] Consult appropriate standard methods for details of collection and preparation of specimens.[5,6]

9- TEST PROCEDURE

The detection of *Salmonella* in foods and other samples of sanitary interest, requires four successive stages: pre-enrichment in non-selective liquid medium, enrichment in one or two selective liquid media, plating out and recognition, confirmation.[5,6]
Allow plates to come to room temperature and to dry the surface of the medium.
From Rappaport Vassiliadis Soy (RVS) Broth (REF 401981) or MSRV Medium (ref 401982) incubated at 41.5 °C ± 1 for 24 h ± 3 h and from MKTT Broth (REF 401745) incubated between 34 °C and 38 °C for 24 h ± 3 h, transfer a loopful of growth on a plate of Chromogenic Salmonella Agar II and on another selective medium for *Salmonella*.
Incubate the inverted plates between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use

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
Salmonella spp.	good growth, magenta-red colonies
Salmonella spp. lac+	good growth, magenta-red or light purple colonies
E. coli	poor growth with colourless colonies
Enterobacter spp.	growth with blue-green colonies or inhibited
Klebsiella spp.	poor growth with blue-green colonies
Pseudomonas spp.	inhibited
Proteus 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, the end user can perform its own Quality Control 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
K. pneumoniae ATCC 700603	35-37°C / 18-24h / A	growth, blue-green colonies
P. aeruginosa ATCC 27853	35-37°C / 18-24h / A	inhibited

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

12 - PERFORMANCES CHARACTERISTICS

Productivity is tested by a quantitative test with 2 target strains: S. Enteritidis ATCC 13076, S. Typhimurium ATCC 14028; CSA II plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 35-37°C for 18-24 hours. The colonies are enumerated on both batches and the productivity ratio (*Pr*) is calculated. If *Pr* is ≥ 0.7 and if the colonies morphology and colour are typical (magenta-red colonies) the results are considered acceptable and conform to the specifications. Furthermore, the productivity characteristics are tested by semi-quantitative ecometric technique with the target strain *S. diarizonae* ATCC 19934 *(*lactose positive strain). After incubation, colonies' colour (light purple colonies) and the amount of growth is evaluated and recorded.

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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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

REF or **REF** Catalogue number	**LOT** 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.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, E-mail: export@biolifeitaliana.it;
web: www.biolifeitaliana.it