

NOVOBIOCIN ANTIMICROBIC SUPPLEMENT (10 mg)

Freeze-dried selective supplement

1 - INTENDED USE

Selective supplement for microbiological culture media for *Salmonella*, *E.coli* O157, *Shigella*.

2 - COMPOSITIONS - (VIAL CONTENTS FOR 500 ML OF MEDIUM)

Novobiocin 10 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Novobiocin Antimicrobial Supplement is a lyophilised antimicrobial compound for use as an additive to culture media for the isolation of *Salmonella* mobile strains^{1,2}, for the preparation of the enrichment broth for *E. coli* O157³ and for the determination of *Shigella*.^{4,5} Novobiocin is an antibiotic designed to target DNA gyrase, a bacterial type IIA topoisomerase and it is active mostly against Gram-positive bacteria but also against a few Gram-negative bacteria.

4- DIRECTIONS FOR MEDIA PREPARATION

MSRV MEDIUM

Suspend 31.6 g of Rappaport Vassiliadis Semisolid Medium Modified (MSRV) REF 401982, in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave. Cool to approximately 47-50°C.

MSRV medium ISO 6579-1 formulation

Add the contents of one vial of Novobiocin Antimicrobial Supplement, reconstituted with 5 mL of sterile purified water. Mix well and pour 15-20 mL into sterile Petri dishes and leave to dry for one hour. Novobiocin concentration in final medium: 10 mg/L

MSRV medium original De Smedt formulation

Add the contents of two vials of Novobiocin Antimicrobial Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Mix well and pour 15-20 mL into sterile Petri dishes and leave to dry for one hour. Novobiocin concentration in final medium: 20 mg/L

SHIGELLA BROTH

Suspend 31.5 g of Shigella Broth Base (REF 402040) in 1000 mL of cold, purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 225 mL in bottles and sterilise by autoclaving at 121°C for 15 minutes. Cool to room temperature.

Dissolve the content of one vial of Novobiocin Antimicrobial Supplement (REF 4240045) with 4 mL of sterile purified water (novobiocin concentration: 2.5 mg/mL). Add a volume of novobiocin solution to the basic medium to obtain the required antibiotic concentration:

Shigella Broth according to ISO 21567 and FDA-BAM for *S. sonnei*: add 50 µL of solution to 225 mL of Shigella Broth Base (final concentration of 0,5 µg/mL broth after 25 g or 25 mL of sample is added).

Shigella Broth according to FDA-BAM for other *Shigella* species: add 300 µL of solution to 225 mL of Shigella Broth Base (final concentration 3 µg/mL broth after 25 g or 25 mL of sample is added).

MODIFIED TRYPTIC SOY BROTH (M-TSB)

Suspend 33 g of Tryptic Soy Broth Modified (mTSB), REF 402155M, in 1000 mL of cold purified water. Mix well and, if necessary, heat slightly to completely dissolve the powder. Distribute 225 mL into flasks of suitable capacity and sterilise by autoclaving at 121°C for 15 minutes. Cool to room temperature and, under aseptic conditions, add to each 225 mL flask, 2.25 mL of Novobiocin Antimicrobial Supplement reconstituted with 5 mL of sterile purified water. Final concentration: 4.5 mg/225 mL or 20 mg/litre.

After reconstitution of the supplement, the remaining novobiocin solution can be stored at 2°C - 8°C for one month.

5 - PHYSICAL CHARACTERISTICS

Freeze-dried supplement appearance short, dense, white pellet
Aspect of the solution colourless, limpid

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Novobiocin Antimicrobial Supplement (10 mg)	Freeze dried supplement	4240045	10 vials, 10 mg/vial

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Basal culture media, autoclave, incubator and laboratory equipment as required, sterile loops and swabs.

8 - SPECIMENS

Food, feed, food chain samples, water, environmental samples.

9 - TEST PROCEDURE

For inoculation, incubation and reading procedures, please refer to the Instructions for Use of dehydrated culture media mentioned above.

10 - 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. Refer to the Instructions for Use of dehydrated culture media mentioned above for the suggested quality control strains.

11 - LIMITATIONS OF THE METHOD

For limitations of the method, please refer to the Instructions for Use of dehydrated culture media mentioned above.

12 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.





- Novobiocin Antimicrobial Supplement is classified as hazardous according to current legislation; consult the Safety Data Sheet before use.
- The supplement and the basal culture media shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the production process of prepared media.
- Novobiocin Antimicrobial Supplement 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 Novobiocin Antimicrobial 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 Sheets of the products are available on the website www.biolifeitaliana.it.
- 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.











13 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at 2-8°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 lyophilized 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). After reconstitution of the supplement, the remaining novobiocin solution can be stored at 2°C - 8°C for one month

14 - REFERENCES

1. De Smedt JM, F Bolderdijk RF, Rappold H, Lautenschlaeger D. Rapid Salmonella Detection in Foods by Motility Enrichment on a Modified Semi-Solid RappaportVassiliadis Medium. J Food Prot. 1986 Jul;49(7):510-514.
2. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp. - ISO 6579-1:2017/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. — Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR/V and SC
3. ISO 16654:2001+AMD 2: 2023. Microbiology of food and animal feeding stuffs -Horizontal method for the detection of Escherichia coli O157-Inclusion of performances testing of all culture media and reagents
4. ISO 21567:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 6: Shigella. Rev. February 2013

TABLE OF APPLICABLE SYMBOLS

 REF or REF Catalogue number	 LOT Batch code	 Use by	 Fragile, handle with care	 Manufacturer
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Store away from direct light	 This side up

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2024/10

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

