MODIFIED SCHOLTENS' AGAR (MSB)

Dehydrated and ready to use culture medium

1 - INTENDED USE

Base medium, in accordance with ISO 10705-2, for the enumeration of somatic coliphages in water.

2 - COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10.00 g
Yeast extract	3.00 g
Meat extract	12.00 g
Sodium chloride	3.00 g
Sodium carbonate	0.75 g
Magnesium chloride ($6H_2O$)	0.60 g
Agar	14.00 g

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

CALCIUM CHLORIDE SOLUTION (1M)
SUPPLEMENTO LIQUIDO – CONTENUTO: 30 mL

Calcium Chloride	3,3 g
Water	30 mL

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Modified Scholtens Broth is prepared in accordance with the formulation proposed by the ISO 10705-2[1] standard for the enumeration of somatic coliphages in water. Somatic coliphages are viruses that belong to the large family of bacteriophages: these are capable of selectively infecting bacteria to replicate, injecting their DNA inside the bacterial cell. Subsequently, the virus begins to multiply inside the bacterium causing its lysis, which will be visible in the culture medium (plaque). Somatic coliphages are indicators of faecal pollution in water, because of their ability to specifically attack the *Escherichia coli* species.
it is a growing medium used as a background medium.
Peptone, yeast extract and meat extract provide the necessary nutrients for *Escherichia coli* (host); sodium chloride, magnesium chloride and calcium chloride maintain osmotic balance without interfering in phage propagation.

4A- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 43.35 g in 1000 mL of cold purified water. Mix thoroughly and warm to completely dissolve the powder. Distribute in tubes or flasks and sterilize by autoclaving at 121°C for 15 minutes. Cool at 45-50°C and add 6 mL of Calcium Chloride Solution (REF. 421020). Dispense 20 mL into 90 mm plates or 50 mL into 150 mm plates.

4B- DIRECTIONS FOR PREPARATION OF READY TO USE FLASKS

Dissolve the contents of the bottle in an autoclave at 100 ± 2°C or in a temperature-controlled water bath at 100°C. Alternatively, the bottle can be placed in a container containing water, which is placed on a heating plate and brought to the boil; loosen the cap slightly before heating. Cool the medium to 45-50°C and add Calcium Chloride Solution (0.6 mL per 100mL of final medium), included in the package. Distribute in sterile plates with aseptic precautions.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Prepared tubes appearance	beige, limpid
Final pH of complete media (at 20-25°C)	7.2 ± 0.5

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Modified Scholtens' Agar Base (MSA)	Dehydrated medium	4017482 4017484	500 g (11.5 L) 5 kg (115)
Modified Scholtens' Agar (MSA)	**Kit:** Ready to use flasks+ Calcium Chloride Solution	511748K2	6 flasks x 100 mL + 2 tubes with 4 mL of Calcium Chloride solution 1M
		511748K3	6 flasks x 200 mL + 2 tubes with 4 mL of Calcium Chloride solution 1M

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, sterile Petri plates, ancillary culture media and reagents.

8 - SPECIMENS

This method, taken from the ISO 10705-2 standard, is suitable for all types of water (natural or waste) and sludge. For collection, transport, storage of samples and culture of the host strain, follow good laboratory practices and refer to the applicable standards (ISO 8199, ISO 5667-1 and ISO 5667-3)[2-4].

9 - TEST PROCEDURE

For the isolation of somatic coliphages and plaque counting, in accordance with ISO 10705-2[1], the following method is recommended:

1. Prepare the host strain as required by the reference standard.
2. Prepare Modified Scholtens's Agar plates as described.
3. Prepare 50 mL of ssMSA (401749) as per IFU (in case of water with high quantity of pollutants, add Nalidixic Acid Solution REF 4240067 to obtain a final concentration of 250mg/L
4. Prepare different dilutions of the water to be treated and dispense 1mL into test tubes, for each dilution (it is recommended to prepare 2 test tubes for each dilution, to make the plaque count more precise)
5. Distribute the dilutions into tubes containing 2.5mL of ssMSA.
6. For each test tube, add 1mL of bacterial culture of the host strain (in the case of highly polluted water, which requires the addition of Nalidixic Acid Solution, use *Escherichia coli* WG5)
7. Distribute the entire contents of the tube on the plates and ensure that the inoculum covers the entire surface

CALCIUM CH
SUPPLEMENT
Calcio Cloru
Acqua

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8. Dry the plates in the incubator with the lid partially open and then close them, invert and incubate at 36 ± 2 °C for 18 ± 2 hours. Do not stack more than six plates.

10 - READING AND INTERPRETATION

Examinate the plates within 4 hours of the end of incubation and perform plaque counts using indirect light. To get an accurate result, consider only plates with a number of plaques between 30 and 300 units. Apply the MPN method.

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.[2]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Escherichia coli ATCC 700078 (WG5)	37 ± 2 °C / 18-24H / A	good growth
Escherichia coli ATCC 13706	37 ± 2 °C / 18-24H / A	good growth

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

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of Modified Scholtens' Agar (TS) is tested for productivity by comparing the results with a previously approved Reference Batch (RB) and with Tryptic Soy Agar (TSA).
Productivity is tested with the quantitative method with the target strains *Escherichia coli* ATCC 700078 (WG5) and *Escherichia coli* ATCC 13706: the plates are inoculated with decimal dilutions of a bacterial suspension in physiological solution and incubated at 37°C for 18-24 hours.
Colonies are counted on the tested batches and the productivity ratio is calculated (Pr: UFC_{MSA}/UFC_{TSA}). If Pr is ≥ 0.7 and if the morphology of the colonies is typical, the results are acceptable and within specifications.

13 - LIMITATIONS OF THE METHOD

- ISO 10705-2 describes how to count plaques, however these can be difficult to see. In this regard, TTC (REF 42111801) can be added to the ssMSA to increase the contrast between the bacterial growth and the plaque-forming unit.
- Mixing the ssMSA with the host strain and the water to be treated could cause the formation of lumps due to the different temperatures of the solutes. Make sure all compounds and reagents are at similar temperatures. If you need to use a bain-marie to dissolve the lumps, set a temperature of 45 ± 1°C and heat for no more than 10 minutes

14 - 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.
- Dehydrated media must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- Apply Good Manufacturing Practice in the production process of prepared media.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest or inhale, avoid the contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- Be careful when opening screw cap flasks to avoid injury from broken glass.
- When using a hot plate and/or double boiler, boil long enough to dissolve the entire medium.
- Wear heat protective gloves during the procedure. Do not place hot bottles in contact with ice or in cold water to speed cooling as this may cause the glass breaking.
- The time required for complete liquefaction of the medium can vary considerably and depends on the actual temperature of the heating device, its power, the size and volume of the bottle.
- Ready-to-use medium in flasks is sterilised by autoclaving.
- 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 and the sterilized tubes inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at 10-30°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).

Ready to use flask

Upon receipt, store in the original packaging at +2°C /+8°C protected from light. In these conditions the bottles are valid until the expiry date indicated on the label. Do not use beyond the expiry date. The bottles removed from the secondary packaging can be used until the expiry date. Opened bottles should be used immediately. Before use, check the closure and integrity of the screw cap. Discard bottles with signs of deterioration (e.g. microbial contamination, abnormal turbidity, atypical color).

The user is responsible for the production and quality control processes of the prepared media and for validating their shelf life, based on the type and conditions of storage (temperature and packaging). According to ISO 10705-2, plates can be stored for no more than 1 month at a temperature of 5 ± 3°C if well protected from dehydration.

16 - REFERENCES

1. ISO 10705-2, Water quality – Detection and Enumeration of bacteriophages, Part 2: enumeration of somatic coliphages
2. ISO 8199:2018 Water quality General requirements and guidance for microbiological examinations by culture.
3. ISO 5667-1:2023 Water quality Sampling Part 1: Guidance on the design of sampling programmes and sampling techniques
4. ISO 5667-3:2024 Water quality Sampling Part 3: Preservation and handling of water samples

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for use	Keep away from direct light	Single use

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First Issue	2024/05
Revision 1	Update of content	2024/12

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