MODIFIED SCHOLTENS' BROTH (MSB)

Dehydrated and ready to use culture medium

1 - INTENDED USE

For the culture and enrichment of host strains, in the methods described by ISO 10705-2

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

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

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.
Modified Scholten's Broth is used for the cultivation and enrichment of somatic coliphages target strains.
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.

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 29.35 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.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Modified Scholtens' Broth (MSB)	Dehydrated medium	4017472 4017474	500 g (17 L) 5 kg (170)
Modified Scholtens' Broth (MSB)	Ready to use flasks	5117472 5117473	6 x 100 mL 6 x 200 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Modified Scholtens' Broth must not be used for the direct inoculation of water samples to test, but for the treatment of the host strain. Follow good laboratory practice and refer to applicable international standards.

9 - TEST PROCEDURE

Standard procedure for setting up host cultures

1. Heat a vial of stock culture, prepared as described in ISO 10705-2[2], until it reaches room temperature and inoculate onto plates of Mac Conkey Agar (REF. 541670), or another equivalent medium based on lactose fermentation, to obtain well isolated colonies. Incubate at 36 ± 2°C for 20 ± 4 hours.
2. Pour 50 ± 5 mL of Modified Scholtens' Broth into a sterile flask and bring to room temperature (growth of the host strain will be faster if the broth is heated to 37°C).
3. Select 3 to 5 lactose positive colonies from the plate and inoculate them into the MSB. Incubate at 36 ± 2°C for 5 ± 1 hours.
4. Measure the bacterial load following the methods described in the standard[3].
5. Add 10mL of sterile glycerol, shake well and dispense approximately 1.2 mL of culture into plastic tubes.
6. Store the tubes in the freezer (-70 ± 10°C) for up to one year. Use host strain cultures as described by ISO 10705-2.

10 - READING AND INTERPRETATION

Microbial growth in Modified Scholtens' Broth is highlighted by the development of turbidity.

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' Broth (TS) is tested for productivity by comparing the results with a previously approved Reference Batch (RB). Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 35-37°C for 18-24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *Escherichia coli* ATCC 700078 (WG5) and *Escherichia coli* ATCC 13706.

13 - LIMITATIONS OF THE METHOD

- Modified Scholtens' Broth is a non-selective medium.
- Host strain culture must be used after preparation. The bacterial load in the working culture must be measured before use, with the aid of a spectrophotometer or following the alternative procedure described in the reference standard[3].
- The host culture tubes, once thawed, must be used during the working day, to avoid an increase in load.

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, 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 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 colour).

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, Modified Scholtens' Broth after autoclaving can be stored in closed containers at +2°C /+8°C for up to 6 months.

16 - REFERENCES

1. ISO 10705-2:2000 , Water quality – Detection and Enumeration of bacteriophages, Part 2: enumeration of somatic coliphages
2. ISO 10705-2:2000 , Water quality – Detection and Enumeration of bacteriophages, Part 2: enumeration of somatic coliphages. 5-6
3. ISO 10705-2:2000 , Water quality – Detection and Enumeration of bacteriophages, Part 2: enumeration of somatic coliphages. 6-7

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

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

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