



Semisolid Modified Scholten's Agar (ssMSA)

M2173I

Intended Use:

Recommended for detection and enumeration of bacteriophages from water samples. The composition and performance criteria are as per specifications laid down in ISO 10705-2:2000.

Composition**

As per ISO 10705-2:2000		Semisolid Modified Scholten's Agar	
Ingredients	g / L	Ingredients	g / L
Peptone	10.000	Peptone	10.000
Yeast extract	3.000	Yeast extract	3.000
Meat Extract	12.000	HM Extract#	12.000
Sodium chloride	3.000	Sodium chloride	3.000
Sodium carbonate (15%)	5.00ml	Sodium carbonate	0.750
Magnesium chloride (100g MgCl ₂ .6H ₂ O in 50ml water)	0.30ml	Magnesium chloride, hexahydrate	0.600
Agar	5.00-10.0	Agar	7.500
pH after sterilization	7.2±0.5	pH after sterilization	7.2±0.5

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat Extract

Directions

Suspend 36.53 grams (the equivalent weight of dehydrated medium per litre) in 1000ml of purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 121±3°C for 15 minutes. Cool to 45-50°C and add 6 ml of filter sterilized solution of 14.6% calcium chloride. If desired add 1 vial of NA Selective Supplement II (FD368) for samples with high bacterial background flora. Allow the medium to set in sloped form with a butt of depth about 2.5cm-5cm.

Principle And Interpretation

The formulation of Scholten's Agar is as described in ISO 10705-2 (1). A coliphage is a type of bacteriophage that infects coliform bacteria such as *Escherichia coli*. Several groups of bacteriophages, amid them the somatic coliphages, have been suggested as indicator micro-organisms in the assessment of faecal contamination of water and food. Somatic coliphages are not only valuable indicators of faecal pollution, but also of the behaviour and survival of human viruses with which they share many basic features (2).

Peptone, HM extract and yeast extract in the medium provides the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium. Magnesium chloride present in the medium raises the osmotic pressure. Sodium carbonate is the essential ion that help to maintain the osmotic balance.

Type of specimen

Water samples

Specimen Collection and Handling

ISO 10705-2:2000 (1)

Rehydrate the content of a lyophilized ampoule of the reference culture of the host strain in 3ml of Modified Scholten's Broth (M2165I) . Transfer the suspension to 50ml of Modified Scholten's Broth (M2165I) and incubate at 36 ± 2°C for (20 ± 4) h. Add 1 ml of inoculum culture to each culture tube containing the aliquots of sample and Semisolid Modified Scholten's Agar (M2173I). Incubate the plates at 36 ± 2°C for (18 ± 2) h. Count the number of plaques on each plate within 4 h after finishing incubation, using indirect oblique light.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber colored clearslightly opalescent gel forms in tubes as slants.

Gelling

Firm, comparable with 0.75% Agar gel

Reaction

Reaction of 3.65% w/v aqueous solution at 25°C. pH after sterilization : 7.2±0.5

pH

6.70-7.70

Cultural Response

Cultural characteristics observed after an incubation at 36 ± 2°C for (20 ± 4) hours

Inoculum	Inoculum (CFU)	Growth
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	good-Luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Water quality — Detection and enumeration of bacteriophages — Part 2: Enumeration of somatic coliphages International Organization for Standardization (ISO), ISO 10705-2:2000.
2. J. Jofre, "Is the replication of somatic coliphages in water environments significant?"; Department of Microbiology, School of Biology, University of Barcelona, Barcelona, Spain.; Journal of Applied Microbiology ISSN 1364-5072.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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