M17 Agar w/ Glycerophosphate

M1063

Intended Use:

Recommended for cultivation of lactic Streptococci and plaque assay of lactic bacteriophages

Composition**

Ingredients	Gms / Litre
Soya peptone	5.000
Biopeptone	5.000
Yeast extract	2.500
HM peptone B #	5.000
Lactose	5.000
Ascorbic acid	0.500
Magnesium sulphate	0.250
Disodium-ß-glycerophosphate	19.000
Agar	10.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 52.25 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

M17 media are based on the formulation described by Terzaghi and Sandine (9) for the cultivation and enumeration of lactic Streptococci and their bacteriophages. It is possible to study plaque morphology and lysogeny. M17 Agar is recommended by the International Dairy Federation (3) for selective enumeration of *Streptococcus thermophilus* from yoghurt. M17 Agar is recommended by APHA for the cultivation of lactic Streptococci (7).

Shankar and Davies (8) reported isolation and enumeration of *Streptococcus thermophilus* from yoghurt. It is also suitable for cultivation and maintenance of starter cultures for cheese and yoghurt manufacturing. This medium helps in detecting streptococcal mutants that are lactose non-fermenters.

Lactic Streptococci are nutritionally fastidious and require complex media for optimal growth (2,6). Disodium glycerophosphate maintains the pH above 5.7. The maintenance of pH is very important as lower pH results in injury and reduced recovery of lactic Streptococci. Glycerophosphate does not form precipitate with calcium which is needed for the plaque assay of lactic bacteriophages.

Soya peptone, yeast extract, HM peptone B and biopeptone provide carbonaceous, nitrogenous compounds, vitamin B complex and other essential growth factors. Lactose is the fermentable carbohydrate and ascorbic acid is stimulatory for the growth of lactic Streptococci. Magnesium sulphate provides essential ions to the organisms. Disodium-ß-glycerophosphate maintains the pH above 5.7. The maintenance of pH is very important as lower pH results in injury and reduced recovery of lactic Streptococci. Disodium glycerophosphate suppresses *Lactobacillus bulgaricus.*

Type of specimen

Dairy samples

Specimen Collection and Handling: (1)

Suggested technique to enumerate streptococci is to seed in mass or by stabbing with agar, melted and cooled to 50-55°C, and incubating them at 42°C for 24 hours period. With these conditions, all the colonies might be streptococci. Longer incubation periods or lower temperatures may cause morphological changes in the colonies, which hinders in the recognition of the colonies. Lactose-positive colonies of streptococci are visible after 15 hours and after 5 days they may reach a diameter of about 3-4 mm, whereas those of lactose-negative are 1 mm in diameter. Bacteriophages presence is observed by appearance of characteristic plaques over the bacterial growth. After use, contaminated materials must be sterilized by autoclaving before discarding.

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.

Limitations

1. The maintenance of pH is very important as lower pH results in injury and reduced recovery of lactic Streptococci.

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
Gelling
Firm, comparable with 1.0% Agar gel.
Colour and Clarity of prepared medium
Light yellow coloured slightly opalescent gel forms in Petri plates
Reaction
Reaction of 5.23% w/v aqueous solution at 25°C. pH : 7.1±0.2
pH
6.90-7.30
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=50%
Lactobacillus bulgaricus ATCC 11842	50-100	none-poor	<=10%
Lactobacillus leichmannii ATCC 4797	50-100	good-luxuriant	>=50%
Lactobacillus plantarum ATCC 8014	50-100	good-luxuriant	>=50%
Streptococcus thermophilus ATCC 14485	50-100	good-luxuriant	>=50%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (4,5).

Reference

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6. Reiter B. and Oran J.D., 1962, J. Dairy Res., 29:63.
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