



## M17 Agar with Glycerophosphate

M1063

M17 Agar with Glycerophosphate is used for cultivation of lactic Streptococci and plaque assay of lactic bacteriophages.

### Composition\*\*

Ingredients	Gms / Litre
Papaic digest of soyaben meal	5.000
Biopeptone	5.000
Yeast extract	2.500
Beef extract	5.000
Lactose	5.000
Ascorbic acid	0.500
Magnesium sulphate	0.250
Disodium - $\beta$ - glycerophosphate	19.000
Agar	10.000
Final pH ( at 25°C)	7.1 $\pm$ 0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 52.25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

### Principle And Interpretation

M17 media are based on the formulation described by Terzaghi and Sandine (1) 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 (5) for selective enumeration of *Streptococcus thermophilus* from yoghurt. M17 Agar is recommended by APHA for the cultivation of lactic Streptococci (6).

Lactic Streptococci are nutritionally fastidious and require complex media for optimal growth (2,3). 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.

Papaic digest of soyabean meal, yeast extract, beef extract 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- $\beta$ -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* @.

Shankar and Davies (4) reported isolation and enumeration of *Streptococcus thermophilus* from yoghurt. M17 Agar is also recommended by the International Dairy Federation (5) for selective 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.

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.

## 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.

### Cultural Response

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

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

## Reference

1. Terzaghi B.E. and Sandine W.E., 1975, Appl. Microbiol., 29:807.
2. Anderson A.W. and Elliker P.R., 1953, J. Dairy Sci., 36:161.
3. Reiter B. and Oran J.D., 1962, J. Dairy Res., 29:63.
4. Shankar P.A. and Davies F.L., 1977, Soc. Dairy Technol., 30:28.
5. International Dairy Federation, 1981, Joint IDF/ISO/AOAC Group E44.
6. Downes F. P. and Ito K. (Eds.), 2001, Compendium of Methods for Microbiological Examination of Food, 4th Ed., APHA, Washington, D.C.

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