

# **Technical Data**

# M17 Agar w/o Lactose

M1019

#### **Intended Use**

On addition of lactose, this medium can be used for cultivation and isolation of lactic Streptococci

# Composition\*\*

Ingredients	Gms / Litre
Tryptone	5.000
Soya peptone	5.000
HM extract #	5.000
Yeast extract	2.500
Ascorbic acid	0.500
Magnesium sulphate	0.250
Disodium - β - glycerophosphate	19.000
Agar	11.000
Final pH ( at 25°C)	$6.9\pm0.2$

<sup>\*\*</sup>Formula adjusted, standardized to suit performance parameters

#### **Directions**

Suspend 48.25 grams in 950 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 and aseptically add 50 ml of 10% w/v lactose solution sterilized separately by autoclaving at 15 lbs pressure (121°C) for 15 minutes or by filtration through a  $0.2\mu m$  membrane filter. Mix well and dispense as desired.

# **Principle And Interpretation**

M17 agar is based on the formulation described by Terzaghi and Sandine (1) for the cultivation and enumeration of lactic Streptococci and their bacteriophages.

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

Tryptone, soya peptone, yeast extract, meat extract provide carbonaceous, nitrogenous compounds, vitamin B complex and other essential growth factors. Lactose is the fermentable carbohydrate. Ascorbic acid is stimulatory for the growth of lactic Streptococci. Magnesium sulphate provides essential ions to the organisms. Diodium  $\beta$ -glycerophosphate maintains the pH about 5.7 due to its buffering action.

Shankar and Davies (4) reported isolation and enumeration of *Streptococcus thermophilus* from yoghurt. Disodium glycerophosphate suppresses *Lactobacillus bulgaricus*. This medium is a standard medium for isolating lactic streptococi (5). M17 Agar is recommended by the International Dairy Federation (6) and ISO Committee (7) for selective enumeration of *Streptococcus thermophilus* from yoghurt. It is also suitable for cultivation and maintenance of starter cultures for cheese and yoghurt manufacturing.

#### Type of specimen

Food and Dairy samples

### **Specimen Collection and Handling**

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8, 9, 10). 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 guidleines should be followed while handling specimens. Saftey guidelines may be referred in individual safety data sheets.

#### **Limitations:**

1. Due to variable nutritional requirements, some strains show poor growth on this medium.

<sup>#</sup> Equivalent to Meat extract

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#### **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 light yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.1% Agar gel.

#### Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 4.83% w/v aqueous solution at 25°C. pH: 6.9±0.2

#### pН

6.70-7.10

#### **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

#### Uvqtcig"cpf"Ujgnh"Nkhg

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 inorder 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (11, 12).

#### 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.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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- 6.International Dairy Federation, 1981, Joint IDF/ISO/AOAC Group E44.
- 7.International Organization for Standardization, 1985, ISO/DIS: 7889.
- 8. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
- 9. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
- 10.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
- 11. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2<sup>nd</sup> Edition.
- 12. 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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