PRODUCT SPECIFICATION SHEET

M17 Agar w/ Glycerophosphate (DM524)

Intended Use
M17 Agar w/ Glycerophosphate (DM524) is recommended for cultivation of lactic *Streptococci* and plaque assay of lactic bacteriophages.

Product Summary and Explanation
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. Lactic streptococci are acid-producing bacteria. They are nutritionally fastidious and require complex culture media for optimum growth. One study showed that in a synthetic medium, all strains had an obligate requirement for at least six amino acids and three vitamins.[2] These homofermentative lactic streptococci produce large amounts of acid and, in a culture medium without an adequate buffering system, the pH decreases and adversely affects growth. Lowrie and Pearce[3] developed M16 Medium but it lacked a strong buffering system. Terzaghi and Sandine[1] worked with M16 Medium and demonstrated that the rapid drop in pH that accompanies lactic streptococcal growth can adversely affect colony size and phage plaque formation. They modified M16 Medium using disodium-ß-glycerophosphate as a buffer and called it M17. 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. Shankar and Davies[4] reported isolation and enumeration of *Streptococcus thermophilus* from yoghurt. 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] 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.

Principles of the Procedure
M17 Agar w/ Glycerophosphate contains papaic digest of soyabean meal, beef extract and biopeptone which provides nitrogen, carbon, amino acids and other essential growth nutrients. Yeast extract is a rich source of vitamin B complex. Lactose is a fermentable carbohydrate. Ascorbic acid is stimulatory for the growth of lactic Streptococci. Magnesium sulphate provides essential ions to the organisms. Disodium glycerophosphate maintains the pH above 5.7.

Formula / Liter

Ingredients	Gms / Liter
Papaic digest of soyabean meal	5.00
Biopeptone	5.00
Yeast extract	2.50
Beef extract	5.00
Lactose	5.00
Ascorbic acid	0.50
Magnesium sulphate	0.25
Disodium - ß – glycerophosphate	19.00
Agar	10.00
Final pH: 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

PRODUCT SPECIFICATION SHEET

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 52.25 grams of the medium in one liter of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well and dispense as desired.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured slightly opalescent gel forms in Petri plates
Reaction of 5.23% solution	pH 7.1 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Enterococcus faecalis ATCC 29212*	50-100	good-luxuriant	>=50%
2.	*Lactobacillus bulgaricus ATCC 11842*	50-100	none-poor	<=10%
3.	*Lactobacillus leichmannii ATCC 4797*		good-luxuriant	>=50%
4.	*Lactobacillus plantarum ATCC 8014*	50-100	good-luxuriant	>=50%
5.	*Streptococcus thermophilus ATCC 14485*	50-100	good-luxuriant	>=50%

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Refer to appropriate references for standard test procedures. 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.
2. Longer incubation periods or lower temperatures may cause morphological changes in the colonies, which hinders in the recognition of the colonies.
3. 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.
4. Bacteriophages presence is observed by appearance of characteristic plaques over the bacterial growth.

Results

Examine plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Storage

Store the sealed bottle containing the dehydrated medium at 10- 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

PRODUCT SPECIFICATION SHEET

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : M17 Agar w/ Glycerophosphate

Product Code : DM524

Available Pack si

References

1. Terzaghi B.E. and Sandine W.E., 1975, Appl. Microbiol., 29:807.
2. Reiter and Oram. 1962. J. Dairy Res. 29:63.
3. Lowrie and Pearce. 1971. J. Dairy Sci. Technol. 6:166.
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.

Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED

Unit 38/39, Kalpataru Industrial Estate,
Near Runwal Estate, Behind 'R-Mall' ,Ghodbunder Raod,
Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

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Prepared By	Checked By	Approved By
01.01.2018	01.01.2018	01.01.2018
Microbiologist	Head Quality Control	Head Quality Assurance

PRODUCT SPECIFICATION SHEET

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