



## Lees Agar

M602

Lees Agar is used for differential enumerations of yoghurt starter bacteria ( *Lactobacillus bulgaricus* and *Streptococcus thermophilus* ).

### Composition\*\*

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Yeast extract	10.000
Lactose	5.000
Sucrose	5.000
Calcium carbonate	3.000
Dipotassium phosphate	0.500
Bromocresol purple	0.020
Agar	18.000
Final pH ( at 25°C)	7.0±0.2

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

### Directions

Suspend 51.52 grams in 1000 ml distilled water. Heat just to boiling and sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes. While dispensing, mix carefully to suspend calcium carbonate evenly. Pour into sterile Petri plates to obtain 4-5 mm thick gel. Note: Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

### Principle And Interpretation

Yoghurt is a fermented milk product in which *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are the essential microbial species that are active in a symbiotic relationship. To obtain optimum consistency, flavour and odour, the two species should be present in about equal numbers in the culture. Dominance by either species can cause defects. Lees Agar, described by Lee et al (1) is used for the differential enumeration of yoghurt starter bacteria. This medium is also recommended by APHA for the same purpose (2). Lees Agar contains sucrose, which most *L. bulgaricus* strains will not ferment, but *S. thermophilus* will, and lactose, which both species utilize. With a suitable combination of sucrose and lactose, the rate of acid production by *S. thermophilus* is enhanced and that of *L. bulgaricus* restricted. Therefore, Streptococci grow first and produce a creamy, buttery aroma from diacetyl and similar metabolites. The redox potential is also thus, lowered by Streptococci, which enables Lactobacilli to grow, thereby growth stimulatory products for Streptococci are synthesized by Lactobacilli. Hence the typical sharp acetaldehyde flavour of mature yoghurt is formed (3).

Casein enzymic hydrolysate and yeast extract provide the essential nitrogenous nutrients to the yoghurt (lactic) starter bacteria. Lactose and sucrose are the fermentable carbohydrates. Calcium carbonate along with dipotassium phosphate is added to buffer the medium and avoid the drastic drop in pH due to lactic acid formation. Bromocresol purple is the pH indicator, which turns yellow in acidic condition and imparts yellow colour to the colony. It is recommended to dry the media plates for 18-24 hours prior to use. Refer appropriate references for standard procedures.

### Quality Control

#### Appearance

Light yellow to light grey homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.8% Agar gel.

#### Colour and Clarity of prepared medium

Purple coloured, opaque gel forms in Petri plates

#### Reaction

Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.0±0.2

**pH**

6.80-7.20

**Cultural Response**

M602: Cultural characteristics observed in presence of Carbon dioxide (CO<sub>2</sub>), after an incubation at 35-37°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<b>Cultural Response</b>				
<i>Lactobacillus bulgaricus</i> ATCC 11842	50-100	luxuriant	>=50%	white
<i>Streptococcus thermophilus</i> ATCC 14485	50-100	luxuriant	>=50%	yellow

**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. Lee S. Y., Vedamuthu E. R., Washam C. J. and Reinbold G. W., 1974, J. Milk Food Technol., 37: 272
2. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
3. Davis J. G., Ashton T. F. and MaCaskill M., 1971, Dairy Ind., 36:569.

Revision : 2 / 2015

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