



TM 2155 – LEE'S MULTIDIFFERENTIAL AGAR

INTENDED USE

Used in the brewing industry for the cultivation and identification of brewing bacteria including fastidious type.

PRODUCT SUMMARY AND EXPLANATION

Lee's Multidifferential Agar is a nutrient medium that detects most organisms commonly found in the brewery. Beer is not a very appropriate medium for the development of bacteria due to its characteristics, such as the low quantity of available nutrients, the presence of alcohol, carbon dioxide and sulphur dioxide, as well as low conservation temperatures. Beer filtration and pasteurization phases also contribute to the stabilization of the product against microorganisms.

COMPOSITION

Ingredients	Gms / Ltr		
Tomato Juice broth	41.000		
Peptonized milk	20.000		
Calcium pantothenate	2.000		
Citric acid	1.100		
Calcium carbonate	5.000		
Polysorbate 80 (Tween 80)	0.500		
Bromocresol green	0.022		
Cycloheximide	0.007		
Agar	15.000		

PRINCIPLE

This medium consists of Tomato juice broth which provides nutrients and acid environment for the growth of acidophilic bacteria. Peptonized milk provides lactose as an energy source. The low pH of the medium inhibits bacteria other than acidophilic bacteria. Polysorbate 80 serves as a source of fatty acids. Bromo cresol green acts as a pH indicator. Acid producing bacteria produce a clear yellow halo around the colonies. Other bacteria produce colonies in colours ranging from colourless to yellow green and blue depending on species and strain. Further tests should be carried out for their identification.

Lactic and acetic acid bacteria are differentiated from non-acid producers by giving a yellow colour to the medium and producing a clear halo zone; Lactobacilli appear translucent to greenish white with dark green center. All lactobacilli have a well-developed halo zone; *Pediococcus* generally produces tiny greenish colonies surrounded by a narrow halo zone; *Acetobacter* produces a weak halo zone, *Acetomonas* produces a substantial halo zone; non-acid producers such as *Flavobacterium, Zymomonas and Enterobacter*) do not produce halo zone or yellow colour in the medium around colonies.

INSTRUCTION FOR USE

- Dissolve 84.63 grams in 1000 ml purified/distilled water.
- Heat the medium just to boiling.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 10 minutes. AVOID OVERHEATING. Stir the medium while dispensing to prevent settling of calcium carbonate.

Note: Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

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QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Light yellow t
Appearance of prepared medium	: Green to light
pH (at 25°C)	: 5.5 ± 0.2

Light yellow to light green homogeneous free flowing powder. Green to light blue coloured opaque gel forms in Petri plates. 5 5 + 0 2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Acinetobacter calcoaceticus	23055	50-100	None-poor	0-10%	25-30°C	48-72 Hours
Lactobacillus acidophilus	4356	50-100	Luxuriant with clear yellow halo	>=70%	25-30°C	48-72 Hours
Lactobacillus fermentum	9338	50-100	Luxuriant with clear yellow halo	>=70%	25-30°C	48-72 Hours
Lactobacillus leichmannii	4797	50-100	Luxuriant with clear yellow halo	>=70%	25-30°C	48-72 Hours
Lactobacillus plantarum	8014	50-100	Luxuriant with clear yellow halo	>=70%	25-30°C	48-72 Hours
Proteus vulgaris	13315	>=10 ³	Inhibited	0%	25-30°C	48-72 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

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PRODUCT DATA SHEET



REFERENCES

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

- 2. 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.
- 3. Mar 1976 DT Journal Article AU Lee, S. Y.; Jangaard, N. O.; Coors, J. H.; Hsu, W. P.; Fuchs, C. M.; Brenner.

4. M. W. PY 1975 AD Adolph Coors Co., Golden, Colorado 80401, USA SO Proceedings. American Society of Brewing Chemists 33.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. *For Lab Use Only Revision: 08 Nov., 2019



