

Technical Information

MRS Broth, Modified (Lactobacillus Heteroferm Screen Broth) (Twin Pack)

Product Code: DM 2164

Application: - Modified MRS Broth is recommended for the isolation and cultivation of *Lactobacillus* species from salad dressings.

Composition**

Ingredients	Gms / Litre
Part A	
Dextrose	20.000
Proteose peptone	10.000
Yeast extract	5.000
Sodium acetate	5.000
Ammonium citrate	2.000
Dipotassium phosphate	2.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Bromocresol green	0.040
Cycloheximide	0.004
Part B	
2-Phenylethyl alcohol	3.000
Final pH (at 25°C)	6.3±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Mayonnise, cooked starch-based dressings are very much similar to mayonnise and pourable dressings available for use. Microorganisms in salad dressings come from the ingredients used in manufacturing equipments and from air. The microflora causing salad dressing to spoil are small in number and consists of few species of *Lactobacillus*, *Saccharomyces* and *Zygosaccharomyces*. MRS Broth, Modified (Lactobacillus Heteroferm Screen Broth) recommended by APHA ⁽¹⁾, is used for the isolation and cultivation of *Lactobacillus* species from salad dressings ⁽²⁾.

MRS Broth, Modified are the modification of MRS medium of deMan et al ⁽³⁾. Proteose peptone and dextrose supply nitrogen, carbon and other elements essential for the growth of Lactobacilli. Polysorbate 80 a mixture of oleic esters, supplies fatty acids required by Lactobacilli. Ammonium citrate, sodium acetate, 2-phenylethyl alcohol and cycloheximide inhibit gram-negative organisms, moulds and certain gram-positive bacteria. Certain yeasts are also suppressed because of presence of cycloheximide. Bromocresol green is the pH indicator, which under acidic conditions, changes colour from green to yellow.

Inoculate 1 ml of 1:10 dilutions of the dressing sample into three MRS Broth, Modified tubes. Incubate at 32°C for 72 ±2 hours. Positive tubes trap CO₂ in the Durhams tubes or bubbles of CO₂ clinging to the inside of the tube and there is a colour change from green to yellow indicating of acid production. These presumptive cultures can be for they confirmed by streaking on MRS Agar, Modified (DM2163) plates.

Methodology

Suspend 47.19 grams of powder media in 1000 ml distilled water containing 1 ml polysorbate 80 and 3g of 2-Phenylethyl alcohol.

Mix thoroughly and dispense in tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If necessary, adjust the pH with glacial acetic acid after sterilization.

Warning: Cycloheximide is very toxic. Avoid skin contact or aerosol formation and inhalation.

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Quality Control

Physical Appearance

Light yellow to bluish grey homogeneous free flowing powder

Colour and Clarity of prepared medium

Green coloured clear to slightly opalescent solution in tubes

Reaction

Reaction of 4.72% w/v aqueous solution at 25°C. pH : 4.3±0.2

pH range

4.10-4.50

Cultural Response/Characteristics

DM 2164: Cultural characteristics observed in presence of 5-10% Carbon dioxide (CO²) , after an incubation at 35-37°C for upto 3 days.

Organism	Inoculum (CFU)	Growth
<i>Lactobacillus acidophilus</i> ATCC 4356	50-100	luxuriant
<i>Lactobacillus fermentum</i> ATCC 9338	50-100	luxuriant
<i>Lactobacillus plantarum</i> ATCC 8014	50-100	luxuriant
<i>Lactobacillus casei</i> ATCC 9595	50-100	luxuriant

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8⁰ in sealable plastic bags for 2-5 days.

Further Reading

1. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.
2. Smittle R. B. and Flowers R. M., 1982, J. Food Protection, 45:977.
3. DeMan J. D., Rogosa M. and Sharpe M. E., 1960, J. Appl. Bacteriol., 23:130.

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