Micro Vitamin Test Culture Agar

M132

Intended Use:

Recommended for cultivation and maintenance of stock cultures of Lactobacilli used in microbiological assays of vitamins.

Composition**

Ingredients	Gms / Litre
Yeast extract	20.000
Peptone	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	2.000
Polysorbate 80 (Tween 80)	0.100
Agar	15.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 52.1 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacillus is a genus of gram-positive facultative anaerobic lactic acid bacteria. The lactic acid bacid bacteria are so named because most of its members convert lactose and other sugars to lactic acid. They are common and usually benign. Many species are prominent in decaying plant material. The production of lactic acid makes its environment acidic which inhibits the growth of some harmful bacteria. Three types of media are generally used in microbiological assays namely maintenance media, inoculum /cultivation media and the test assay media.

Micro Vitamin Test Agar is used for carrying stock cultures of Lactobacilli and other test organisms used in microbiological assays (1). This media can also be used for routine cultivation of Lactobacilli in microbiological assays of vitamins and in inoculum preparation for assays.

Peptone and yeast extract in the medium provide nitrogen, sulphur, vitamins and other essential nutrients for growth. Dextrose is the energy source. Polysorbate 80 is the fatty acid source. Potassium dihydrogen phosphate buffers the medium.

Stock cultures are prepared by stab inoculation in triplicates. One is used for preparation of stock cultures while others are used for inoculum preparation for assays. Transfer of cultures should be made at weekly or biweekly intervals.

Suspend a 16-24 hours culture of Lactobacilli from Micro Vitamin Test Culture Agar into Micro Vitamin Test Inoculum Broth. After an incubation at 35-37°C for 18-24 hours, centrifuge the culture and decant the supernatant. Re-suspend the centrifuged cells in 10 ml of sterile saline suspension. Repeat the washing two more times. Dilute the washed cell suspension with basal assay medium or as desired to obtain the required density of cells.

For procedure of Vitamin Assay, refer standard references (2).

Type of specimen

Isolated Microorganism

Specimen Collection and Handling:

Stock cultures are prepared by stab inoculation in triplicates. One is used for preparation of stock cultures while others are used for inoculum preparation for assays.

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 guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Transfer of cultures should be made at weekly or biweekly intervals.

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 yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 5.2% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Lactobacillus casei ATCC 9595	50-100	good-luxuriant
Lactobacillus viridescens ATCC 12706	50-100	good-luxuriant
Lactobacillus leichmanni ATCC 4797	50-100	good-luxuriant
Lactobacillus plantarum ATCC 8014	50-100	good-luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order 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. Use before expiry date on the label.
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 (3,4).

Reference

1. Atlas R. M., 1993, Handbook of Microbiological Media, Parks L.C., (Ed.), CRC Press, Inc.

2. Horwitz, (Ed.), 2000, Official Methods of Analysis of AOAC International, 17th Ed., Vol. I, AOAC International, Gaithersburg, Md.

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

4. 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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