



Micro Vitamin Test Inoculum Broth

M133

Micro Vitamin Test Inoculum Broth is recommended for preparation of inocula of *Lactobacilli* used in microbiological assays of vitamins.

Composition**

Ingredients	Gms / Litre
Yeast extract	20.000
Proteose peptone	5.000
Dextrose	10.000
Monopotassium phosphate	2.000
Polysorbate 80	0.100
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.1 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Lactobacillus is a genus of gram-positive facultative anaerobic lactic acid bacteria. The lactic acid 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 Inoculum Broth 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.

Proteose 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. Monopotassium 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).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution in tubes

Reaction

Reaction of 3.71% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural Response

M133: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
<i>Lactobacillus casei</i> ATCC 9595	50-100	good-luxuriant
<i>Lactobacillus leichmanni</i> ATCC 7830	50-100	good-luxuriant
<i>Lactobacillus plantarum</i> ATCC 8014	50-100	good-luxuriant
<i>Lactobacillus viridescens</i> ATCC 12706	50-100	good-luxuriant

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. 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.

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