



## B12 Culture Agar (*L. leichmannii* Maintenance Medium)

M035

B12 Culture Agar is recommended for the propagation, cultivation and maintenance of *Lactobacillus leichmannii* ATCC 7830 used as the test organism in the Vitamin B12 Assay.

### Composition\*\*

Ingredients	Gms / Litre
Peptic digest of animal tissue	7.500
Yeast extract	7.500
Dextrose	10.000
Monopotassium phosphate	2.000
Tomato juice (from 100 ml)	5.000
Polysorbate 80	0.100
Agar	10.000
Final pH ( at 25°C)	6.8±0.2

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

### Directions

Suspend 42.1 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in 10 ml amounts in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubed medium in an upright position with rapidity to avoid colour formation due to overheating.

### Principle And Interpretation

B12 Culture Agar recommended by USP for cultivation and maintenance of *Lactobacillus leichmannii* ATCC 7830 (*Lactobacillus delbrueckii subsp.lactis* ATCC 7830) which is used as a test bacterium during the microbiological estimation of vitamin B12 (1). *Lactobacillus* species have very exacting nutritional requirements for amino acids and vitamins. This restricts them to nutritionally compete in the environment. *Lactobacillus* species grow poorly on non-selective media. Kulp (2) found that the growth of *Lactobacillus acidophilus* was enhanced with tomato juice, while investigating the use of tomato juice on bacterial development, which was reported earlier by Mickle and Breed (3) for the microbiological assay of vitamins.

Peptic digest of animal tissue serves as a source of nitrogen and amino acids. Yeast extract is the vitamin source. Tomato juice is added to create the proper acidic environment. Dextrose is the carbon source and Polysorbate 80 acts as an emulsifier. Monopotassium phosphate provides buffering capacity.

Stock cultures of *Lactobacillus leichmannii* ATCC 7830 are prepared by stab inoculation of 3 or more tubes. These stab cultures are made at least 3 times in a week. Do not use the culture for preparing assay inoculum if it is over 4 days old. Before using a fresh culture for assay, make at least 10 successive transfers of the culture in 15 days period. Incubate the culture for 16-24 hours at 35°C but hold constant within 0.5°C. After incubation, store at 2-8°C.

### Quality Control

#### Appearance

Cream to yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.0% Agar gel.

#### Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent gel forms in tubes.

#### Reaction

Reaction of 4.21% w/v aqueous solution at 25°C. pH : 6.8±0.2

#### pH

6.60-7.00

#### Cultural Response

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M035: Cultural characteristics observed after an incubation at 35-37°C for 16-24 hours.

<b>Organism</b>	<b>Inoculum (CFU)</b>	<b>Growth</b>
<i>Lactobacillus leichmannii</i> ATCC 7830	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. The United States Pharmacopoeia, 2006, USP 29/ NF 24, The United States Pharmacopoeial Convention, Rockville, MD.
2. Kulp and White, 1932, Science 76:17.
3. Mickle and Breed, 1925, Technical Bulletin 110, NY State Agriculture Ex. station, Geneva, N.Y.

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#### Disclaimer :

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