



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

M035

Intended Use:

Recommended for propagation, cultivation and maintenance of *Lactobacillus leichmannii* ATCC 7830.

Composition**

| Ingredients | Gms / Litre |
|--------------------------------|-------------|
| Peptone | 7.500 |
| Yeast extract | 7.500 |
| Dextrose (Glucose) | 10.000 |
| Potassium dihydrogen phosphate | 2.000 |
| Tomato juice (from 100 ml) | 5.000 |
| Polysorbate 80 (Tween 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 purified / 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 (5). *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 (3) 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 (4) for the microbiological assay of vitamins.

Peptone serves as a source of nitrogen, carbon and long chain 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. Potassium dihydrogen 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.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling

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.

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. Overheating may result in darkening of the medium.

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 1.0% 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

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

| Organism | Inoculum (CFU) | Growth |
|---|----------------|----------------|
| <i>Lactobacillus leichmannii</i> ATCC 7830 | 50-100 | good-luxuriant |

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (1,2).

Reference

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. Kulp and White, 1932, Science 76:17.
4. Mickle and Breed, 1925, Technical Bulletin 110, NY State Agriculture Ex. station, Geneva, N.Y.
5. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention, Rockville, MD.

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Disclaimer :

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