# Lactobacilli Broth, AOAC

**M367**

## Intended Use:

Recommended for preparation of inocula of test bacteria used in microbiological assays of B vitamins.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Peptonized SM powder# | 15.000 |
| Yeast extract | 5.000 |
| Dextrose (Glucose) | 10.000 |
| Tomato juice (100 ml) | 5.000 |
| Potassium dihydrogen phosphate | 2.000 |
| Polysorbate 80 (Tween 80) | 1.000 |
| Final pH ( at 25°C) | 6.8±0.2 |

**Formula adjusted, standardized to suit performance parameters
# Equivalent to Peptonized milk

## Directions

Suspend 38 grams in 1000 ml purified/distilled water. Heat, if necessary, to boiling to dissolve the medium completely. Distribute into tubes in 10 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

## Principle And Interpretation

Lactobacilli Broth, AOAC was formulated by Loy (4) and recommended by AOAC (5) for preparing inocula of test bacteria used for Microbiological assay of Vitamin B. Stock cultures of *Lactobacillus leichmanni* ATCC 7830, *Lactobacillus plantarum* ATCC 8014, *Lactobacillus casei* ATCC 7469, *Enterococcus hira*e ATCC 8043 and other such B vitamin requiring strains are prepared by stab inoculation of sterile Lactobacillus Agar, AOAC and incubated for 18-24 hours at a constant temperature between 30-40°C. Lactobacilli Broth, AOAC is used for cultivation and preparation of inocula of the above mentioned stock cultures (1). Inoculum is prepared by inoculating these cultures in Lactobacillus Broth, AOAC and incubating at 35-37°C. Peptonized SM powder and yeast extract provide essential growth nutrients. Dextrose is the energy source. Phosphate provides buffering system while tomato juice helps in lowering the pH. Polysorbate 80 supplies fatty acids. Before using a culture in any assay, at least 2 successive transfers during a 1-2 week period are essential. Any culture older than one week should not be used.

## Type of specimen

Isolated Microorganism

## Specimen Collection and Handling

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. Any culture older than one week should not be used.

## 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

**Colour and Clarity of prepared medium**
Medium amber coloured clear solution in tubes

**Reaction**
Reaction of 3.8% 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 fo r 18 - 48 hours

| Organism | Inoculum (CFU) | Growth |
|---|---|---|
| *Enterococcus hirae* ATCC 8043 (00089*) | 50-100 | luxuriant |
| *Lactobacillus casei* ATCC 7469 (00101*) | 50-100 | luxuriant |
| *Lactobacillus leichmannii* ATCC 7830 | 50-100 | luxuriant |
| *Lactobacillus plantarum* ATCC 8014 | 50-100 | luxuriant |

Key : (*) Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

## Reference

1. Atlas R. M., 2004, Handbook of Microbiological Media, 3rd Edition, CRC Press
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.
4. Loy, 1958, J. AOAC, 4:61.
5. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.

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Disclaimer :

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