



Acetobacter Agar w/ HL Extract

Intended Use:

Recommended for maintenance of glucose positive Acetobacter species.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
HL extract #	2.000
Dextrose (Glucose)	20.000
Calcium carbonate	10.000
Agar	20.000
Final pH (at 25°C)	7.4±0.2
**Formula adjusted, standardized to suit performance parameters	

Equivalent to Liver extract

Directions

Suspend 57 grams in 1000 ml purified / distilled water. Heat just to boiling. Dispense in test tubes, taking care to distribute calcium carbonate evenly. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Shake the tubes and place them to cool in a slanted position so as to keep the calcium carbonate in suspension.

Note: Due to presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

Principle And Interpretation

Acetobacter species are aerobic, gram-negative organisms. Acetic acid bacteria are found in fruits with high carbohydrate concentration, which is selective for yeasts, that produce ethanol. This ethanol forms the substrate for acetic acid bacteria and may oxidize ethanol to acetic acid (6). Various synthetic and maintenance media for *Acetobacter* cultures have been cited (1). A typical maintenance medium is Acetobacter Agar (1) Acetobacter Agar is formulated as per Manual of Microbiological

Methods (5) and used for the maintenance of *Acetobacter* species utilizing glucose or mannitol (2). Acetobacter Agar w/ liver extract is a modification of Acetobacter Agar.

Tryptone, HL extract in the medium provides nitrogen, vitamins and minerals necessary to support bacterial growth. Glucose acts as energy source. Calcium carbonate acts as a buffer.

Type of specimen

Food samples - fruits

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6). 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. Further biochemical and serological tests must be carried out for complete identification.
- 2. Some organism may show poor growth due to nutritional variation.

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.

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Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Light amber coloured opalescent gel with heavy white precipitate, forms in tubes as slants.

Reaction

Reaction of 5.7% w/v aqueous solution at 25°C. pH : 7.4±0.2

pН

7.20-7.60

Cultural Response

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

Organism	Inoculum (CFU)
Acetobacter aceti ATCC 15973	50-100
Acetobacter liquifaciens ATCC 14835	50-100

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

- 1. Asai, 1968, Univ. of Tokyo Press, Tokyo, Japan and Univ. Park Press, Baltimore, MD.
- 2. Catalogue of Bacteria and Bacteriophages, 1992, 18th Ed., American Type Culture Collection, Rockville, 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.
- 5. Manual of Microbiological Methods, 1957, Society of American Bacteriologists, McGraw-Hill Book Company, New York.
- 6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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