



Acetamide Broth (Twin Pack)

M148

Intended Use:

Recommended for confirmation of *Pseudomonas aeruginosa* in water samples.

Composition**

Ingredients	Gms / Litre
Part A	-
Acetamide	10.000
Part B	-
Sodium chloride	5.000
Dipotassium hydrogen phosphate	1.390
Potassium dihydrogen phosphate	0.730
Magnesium sulphate	0.500
Phenol red	0.012
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 7.63 grams of part B in 1000 ml purified / distilled water. Add 10.0 grams of Part A. Heat if necessary to dissolve the medium completely. Dispense in 10ml amounts in tubes or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

Principle And Interpretation

Acetamide Broth is formulated as per the recommendation of Standard Methods for the Examination of Water and Wastewater (1). Acetamide is utilized by a wide variety of non-fermenting organisms (2,3). The media contains inorganic salts and acetamide a sole carbon and nitrogen source. However very few organisms growing in the medium metabolize acetamide by the process of deamination (acrylamidase activity) (4,5). This unique ability is useful in identification of various non-fermenting gram-negative organisms (6,7,8). This ability is shown by *Pseudomonas aeruginosa*, *Pseudomonas aciovorans* Group III (*Achromobacter xylosoxidans*) and *Alcaligenes odorans* (9). Acetamide deamination leads to the liberation of ammonia, which thereby increases the pH of the medium, leading to a subsequent colour change of the phenol red indicator from yellow orange to purplish red. Some strains require upto seven days to exhibit a positive reaction as they deaminate acrylamide slowly. However, only about 40% of apyocyanogenic strains of *Pseudomonas aeruginosa* exhibit a positive reaction. It is therefore, not advisable to rely on this test as the only criterion for identification. Phosphates in the media serve as buffering agents, Magnesium sulphate is a source of ions that stimulate metabolism whereas Acetamide serves as the sole nitrogen and carbon source. Sodium chloride maintains osmotic equilibrium. Phenol red is the pH indicator.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (10). 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. Only about 40% of apyocyanogenic strains of *Pseudomonas aeruginosa* exhibit a positive reaction. It is therefore, not advisable to rely on this test as the only criterion for identification.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

Part A : Colourless deliquescent crystals Part B : Light yellow to light pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Orange coloured clear solution in tubes

Reaction

Reaction of the medium (Mixture of 1% w/v Part A and 0.76% w/v of Part B) at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 4-7 days.

Organism	Inoculum (CFU)	Growth	Deamination
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	good - luxuriant	positive reaction, purplish red colour (within 7days)
<i>Stenotrophomonas maltophilia</i> ATCC 13637	50-100	good - luxuriant	negative reaction, no purplish red colour (after 7 days)

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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. 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 (11,12).

Reference

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