



Acetamide Broth (Twin Pack)

M148

Acetamide Broth is recommended for confirmation *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 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.

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.

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

M148: Cultural characteristics observed after an incubation at 35-37°C for 4-7 days.

| Organism | Inoculum (CFU) | Growth | Deamination |
|----------|-------------------|--------|-------------|
|----------|-------------------|--------|-------------|

Cultural Response

| | | | |
|--|--------|------------------|---|
| <i>Pseudomonas aeruginosa</i> ATCC 27853 | 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) |

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

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3. Stainier, Palleroni and Doudoroff, 1966, J. Gen Microbiol., 43:159.
4. Pickett M. J. and Pedersen M.M., 1970, Can. J. Microbiol., 16:351.
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6. Hedberg, 1969, Appl. Microbiol., 17: 481
7. Smith and Dayton, 1972, Appl. Microbiol., 24: 143
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