Acetamide Broth (Twin Pack)

M148I

Acetamide Broth is recommended for confirmation of non-fermentative gram-negative bacteria, particularly *Pseudomonas aeruginosa* .

Composition**

Ingredients	Gms / Litre
Part A	-
Acetamide	2.000
Part B	-
Sodium chloride	0.200
Potassium dihydrogen phosphate	1.000
Magnesium sulphate anhydrous	0.200
Iron sulphate	0.0005
Sodium molybdate	0.005
Final pH (at 25°C)	7.0±0.5

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 1.4 grams of part B in 1000 ml distilled water. Add 2 grams of Part A. Heat if necessary to dissolve the medium completely. Dispense in tubes or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

A wide variety of pathogenic microorganisms can be transmitted to humans through use of natural fresh and marine recreational waters contaminated by waste water (1, 2). *Pseudomonas aeruginosa* is one of the organisms that are capable of growth in water at very low concentrations of nutrients. While the primary indicators of water quality are *Escherichia coli* and *Enterococci* , the enumeration of *Pseudomonas aeruginosa* in recreational waters may be useful in cases of discharge of pulp and paper wastes and effluents from textile finishing plants into receiving waters. One of the unique properties of *P. aeruginosa* is its ability to produce ammonia from acetamide.

Acetamide Broth, formulated as per DRAFT prEN 12780:1999 is recommended for the confirmation of non-fermentative gram-negative *Pseudomonas aeruginosa* (3). Organisms growing in this medium metabolize acetamide by process of deamination (acrylamidase activity) (4, 5). This ability is shown by *Ps. aeruginosa* , *Ps. acidovorans* Group III (*Achromobacter xylosoxidans*) and *Alcaligens odorans* (6).

Acetamide in the medium serves as a sole source of nitrogen and carbon. Magnesium sulphate, sodium molybdate and iron sulphate are the sources of ions that stimulate metabolism. Phosphate serves as a buffering agent.

The test water samples are filtered through sterile cellulose ester membrane filters. These filters are aseptically placed on Pseudomonas Agar Base (M085) containing Cetrinix Supplement (FD029). These plates with filters are incubated at 35- 37°C for 24-48 hours. Pyocyanin-producing colonies are counted as confirmed *Ps.aeruginosa* . Non-pyocyanin- producing fluorescent colonies are counted as presumptive *Ps.aeruginosa* . These presumptive *Ps.aeruginosa* colonies are confirmed by using Acetamide Broth (M148I)(7). Production of ammonia from acetamide can be detected by the addition of Nesslers reagent (R010).

Quality Control

Appearance

Part A : Colourless deliquescent crystals Part B : Off white to white homogeneous free flowing powder

Colour and Clarity of prepared medium

Colourless clear solution

Reaction

Reaction of complete medium (mixture of 0.2% w/v Part A and 0.14% w/vof Part B) at 25°C. pH : 7.0±0.5

pH
6.50-7.50

Cultural Response
M148I: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Deamination
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	positive, yellow to brick red colour formation on addition of Nessler's reagent (R010)
Stenotrophomonas maltophilia ATCC 13637	50-100	good-luxuriant	negative, no colour formation on addition of Nessler's reagent R010)

Storage and Shelf Life
Store below 30ºC in tightly closed container and the prepared medium at 2-8º C. Use before expiry date on the label.

Reference
1. Cabelli V. J., 1980, U. S. Environmental Protection Agency, Research Triangle Park, N.C.
2. Dufour A. P., 1984, U. S. Environmental Protection Agency, Research Triangle Park, N.C
3. Directive of Council of the European Union, Draft prEN 12780:1999
4. Pickett M. J. and Pedersen M. M., 1970, Can. J. Microbiol., 16:351.
5. Pickett M. J. and Pedersen M. M., 1970, Can. J. Microbiol., 16:401.
6. Oberhofer and Rowen, 1974, Appl. Microbiol., 28:720.
7. International Organisation for Standardization(ISO),2006,Draft ISO/DIS,16266

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